# WEST Search History

DATE: Tuesday, December 10, 2002

| Set Name side by side | Query | Hit Count | Set Name result set |
|---|---|---|---|
| *DB=USPT; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | | |
| L7 | l1 and L6 | 3 | L7 |
| L6 | p450 with la | 14 | L6 |
| L5 | (p450 near 1a).ab. | 0 | L5 |
| L4 | l1 and l2 | 75 | L4 |
| L3 | l1 andL2 | 0 | L3 |
| L2 | p450.ab. | 86 | L2 |
| L1 | cytochrome.ab. | 199 | L1 |

END OF SEARCH HISTORY

# WEST Search History

DATE: Tuesday, December 10, 2002

| Set Name side by side | Query | Hit Count | Set Name result set |
|---|---|---|---|
| | *DB=USPT; THES=ASSIGNEE; PLUR=YES; OP=ADJ* | | |
| L12 | L11 and (retinoic or retinoid or retinol$) | 1 | L12 |
| L11 | cyp-1a or cyp1a or (cytochrome p450 1a) | 10 | L11 |
| L10 | cyp-1a or cyp1a or (chtochrome p450 1a) | 7 | L10 |
| L9 | (l1-l8) and (cytochrome or cyp1a) | 0 | L9 |
| L8 | l7 same (retinoid or retinoic or retinol) | 14 | L8 |
| L7 | isoflavone | 373 | L7 |
| L6 | L5 same acne | 4 | L6 |
| L5 | l4 same (retinoid or retinoic or retinol) | 43 | L5 |
| L4 | kaempferol or terpineol or napthoflavone or hesperetin or quercetin | 4305 | L4 |
| L3 | genistein same (retinoid or retinoic or retinol) same acne | 0 | L3 |
| L2 | genistein same (retinoid or retinoic or retinol)same acne | 0 | L2 |
| L1 | genistein same (retinoid or retinoic or retinol) | 25 | L1 |

END OF SEARCH HISTORY

L24 ANSWER 18 OF 18 CAPLUS COPYRIGHT 2002 ACS
AN 2002:521422 CAPLUS
DN 137:83423
TI Skin care product containing retinoids, retinoid booster and phytoestrogens in a dual compartment package
IN Pillai, Sreekumar; Granger, Stewart Paton; Scott, Ian Richard; Pocalyko, David Joseph
PA Unilever P.L.C., UK; Unilever N.V.; Hindustan Lever Limited
SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K007-00
CC 62-4 (Essential Oils and Cosmetics)
Section cross-reference(s): 63
FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2002053108 | A2 | 20020711 | WO 2001-EP14486 | 20011206 |
| | WO 2002053108 | A3 | 20020926 | | |
| | W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| | RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| | US 2002143059 | A1 | 20021003 | US 2001-3850 | 20011102 |
| PRAI | US 2000-258457P | P | 20001228 | | |

AB A stable skin care product contains a first compn. comprising 0.001-10% a retinoid, a second compn. comprising 0.0001-50% at least 1 retinoid booster and 0.001-10% a phytoestrogen. The products also contain a compartment for storing the first compn. and a second compartment for storing the second compn., the first and second compartments being joined together. Synergy between **genistein** and daidzein and **retinoids** was tested. In both the studies **genistein** was delivered to the cells in a sol. form in DMSO/EtOH. **Genistein** (1 .mu.m) alone stimulated CRABP-2 significantly. Both **genistein** and daidzein stimulate **retinoid** activity in a synergistic manner. All the **retinoids** tested, except retinyl acetate showed synergy with **genistein** and daidzein. These data support our claim that the phytoestrogenic flavonoids **genistein** and daidzein, when supplied to cells in a sol. form, synergistically enhanced the activity of **retinoids**.

ST skin retinoid phytoestrogen dual compartment package
IT Skin, disease
(aging, wrinkles; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)
IT Amides, biological studies
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(castor oil, N-(hydroxyethyl); skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)
IT Amides, biological studies
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(coco, N-(hydroxyethyl); skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)
IT Skin, disease
(dry; skin care product contg. retinoid boosters and phytoestrogens in

dual compartment package)

IT Skin
(epidermis; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Skin, disease
(photoaging; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Estrogens
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(phytoestrogens; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT **Acne**
Animal tissue culture
Cosmetics
Fibroblast
Sebum
(skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Flavonoids
Linseed oil
Retinoids
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Cosmetics
(skin-lightening; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Skin
(stratum corneum; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT Drug delivery systems
(topical; skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

IT 59-31-4, 2-Hydroxyquinoline 60-33-3, Linoleic acid, biological studies 68-26-8, Retinol 77-52-1, Ursolic acid 78-70-6, Linalool 79-81-2, Retinyl palmitate 80-73-9, 1,3-Dimethyl-2-imidazolidinone 91-64-5, Coumarin 97-78-9, N-Laurylsarcosine 106-22-9, Citronellol 106-24-1, Geraniol 117-39-5, Quercetin 127-41-3, .alpha.-Ionone 127-47-9, Retinyl acetate 148-24-3, 8-Hydroxyquinoline, biological studies 302-79-4, Retinoic acid 446-72-0, **Genistein** 471-53-4, 18.beta.-Glycyrrhetinic acid 480-41-1, Naringenin 486-66-8, Daidzein 544-31-0, Palmitic acid monoethanolamide 631-89-0, Retinyl linoleate 695-10-3D, cocoyl derivs. 871-37-4, Oleyl betaine 4602-84-0, Farnesol 5392-40-5, Citral 16058-19-8 22916-47-8, Miconazole 38083-17-9, Climbazole 56863-02-6 65277-42-1, Ketoconazole 68171-52-8, Linoleic acid monoethanolamide 80111-68-8, Damascone 112708-19-7, 1H-Benzotriazolamine 124753-97-5 159065-21-1 386704-13-8, Utrecht-2
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(skin care product contg. **retinoid** boosters and phytoestrogens in dual compartment package)

=>

L25 ANSWER 1 OF 16 USPATFULL

ACCESSION NUMBER: 2002:235072 USPATFULL

TITLE: Skin care product containing retinoids and phytoestrogens in a dual compartment package

INVENTOR(S): Pillai, Sreekumar, Wayne, NJ, UNITED STATES
Granger, Stewart Paton, Paramus, NJ, UNITED STATES
Scott, Ian Richard, Allendale, NJ, UNITED STATES
Pocalyko, David Joseph, Wayne, NJ, UNITED STATES

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 2002127255 | A1 | 20020912 | |
| APPLICATION INFO.: | US 2001-36589 | A1 | 20011107 | (10) |

| | NUMBER | DATE | |
|---|---|---|---|
| PRIORITY INFORMATION: | US 2000-258456P | 20001228 | (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: UNILEVER, PATENT DEPARTMENT, 45 RIVER ROAD, EDGEWATER, NJ, 07020
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
LINE COUNT: 497
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L37 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS

IN **Pillai, Sreekumar**; Granger, Stewart Paton; Scott, Ian Richard; Pocalyko, David Joseph

AB A stable skin care product contains a first compn. comprising 0.001-10% a retinoid, a second compn. comprising 0.0001-50% at least 1 retinoid booster and 0.001-10% a phytoestrogen. The products also contain a compartment for storing the first compn. and a second compartment for storing the second compn., the first and second compartments being joined together. Synergy between **genistein** and daidzein and retinoids was tested. In both the studies **genistein** was delivered to the cells in a sol. form in DMSO/EtOH. **Genistein** (1 .mu.m) alone stimulated CRABP-2 significantly. Both **genistein** and daidzein stimulate retinoid activity in a synergistic manner. All the retinoids tested, except retinyl acetate showed synergy with **genistein** and daidzein. These data support our claim that the phytoestrogenic flavonoids **genistein** and daidzein, when supplied to cells in a sol. form, synergistically enhanced the activity of retinoids.

IT 59-31-4, 2-Hydroxyquinoline 60-33-3, Linoleic acid, biological studies 68-26-8, Retinol 77-52-1, Ursolic acid 78-70-6, Linalool 79-81-2, Retinyl palmitate 80-73-9, 1,3-Dimethyl-2-imidazolidinone 91-64-5, Coumarin 97-78-9, N-Laurylsarcosine 106-22-9, Citronellol 106-24-1, Geraniol 117-39-5, Quercetin 127-41-3, .alpha.-Ionone 127-47-9, Retinyl acetate 148-24-3, 8-Hydroxyquinoline, biological studies 302-79-4, Retinoic acid 446-72-0, **Genistein** 471-53-4, 18.beta.-Glycyrrhetinic acid 480-41-1, Naringenin 486-66-8, Daidzein 544-31-0, Palmitic acid monoethanolamide 631-89-0, Retinyl linoleate 695-10-3D, cocoyl derivs. 871-37-4, Oleyl betaine 4602-84-0, Farnesol 5392-40-5, Citral 16058-19-8 22916-47-8, Miconazole 38083-17-9, Climbazole 56863-02-6 65277-42-1, Ketoconazole 68171-52-8, Linoleic acid monoethanolamide 80111-68-8, Damascone 112708-19-7, 1H-Benzotriazolamine 124753-97-5 159065-21-1 386704-13-8, Utrecht-2
RL: COS (Cosmetic use); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(skin care product contg. retinoid boosters and phytoestrogens in dual compartment package)

ACCESSION NUMBER: 2002:521422 CAPLUS
DOCUMENT NUMBER: 137:83423
TITLE: Skin care product containing retinoids, retinoid booster and phytoestrogens in a dual compartment package
INVENTOR(S): **Pillai, Sreekumar**; Granger, Stewart Paton; Scott, Ian Richard; Pocalyko, David Joseph
PATENT ASSIGNEE(S): Unilever P.L.C., UK; Unilever N.V.; Hindustan Lever Limited
SOURCE: PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002053108 | A2 | 20020711 | WO 2001-EP14486 | 20011206 |
| WO 2002053108 | A3 | 20020926 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,

BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
US 2002143059 A1 20021003 US 2001-3850 20011102
PRIORITY APPLN. INFO.: US 2000-258457P P 20001228

=>

L37 ANSWER 4 OF 6 USPATFULL

IN **Pillai, Sreekumar**, Wayne, NJ, United States

SUMM Over 500 compounds present in plants have been described to have estrogenic activity. These compounds, collectively called phytoestrogens, are found in a diverse number of plants including cereals, legumes (including chick peas) and grasses (Price et al., Naturally occurring estrogens in foods--a review., Food additives and contaminants., 2, p. 73-106, 1985). Their concentrations vary in the different parts of the plants, geographical locations, year of growth etc. Two major classes of plant compounds which possess phytoestrogenic activitv are flavonoids and coumestans. Some of the commonly described phytoestrogenic compounds are **genistein**, biochanin A, formononetin, daidzein and their glycoside derivatives (Knight et al., Phytoestrogens-a short review., Maturitas, J. Climactreic and post-menopause, 22, p.167-75, 1995).

| | |
|---|---|
| ACCESSION NUMBER: | 2000:24294 USPATFULL |
| TITLE: | Skin care compositions containing an organic extract of chick pea |
| INVENTOR(S): | **Pillai, Sreekumar**, Wayne, NJ, United States<br>Santhanam, Uma, Tenafly, NJ, United States<br>Carlomusto, Marieann, Palisades Park, NJ, United States<br>Bosko, Carol, Oradell, NJ, United States |
| PATENT ASSIGNEE(S): | Chesebrough-Pond's USA Co., Division of Conopco, Inc., Greenwich, CT, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 6030620 | | 20000229 | |
| APPLICATION INFO.: | US 1997-901052 | | 19970725 | (8) |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |
| PRIMARY EXAMINER: | Page, Thurman K. | | | |
| ASSISTANT EXAMINER: | Berman, Alysia | | | |
| LEGAL REPRESENTATIVE: | Mitelman, Rimma | | | |
| NUMBER OF CLAIMS: | 4 | | | |

L37 ANSWER 3 OF 6 USPATFULL

IN **Pillai, Sreekumar**, Wayne, NJ, United States

SUMM Phytoestrogens are natural compounds which have estrogen-like activity and which are found in plants. Some bioflavonoids, such as **genistein** and daidzein, are known phytoestrogens. WO 99/04747 (Unilever) teaches that resveratrol, a compound found in a variety of plants, is a phytoestrogen and discloses cosmetic compositions containing resveratrol. One of the disclosed compositions also includes retinyl palmitate.

| | |
|---|---|
| ACCESSION NUMBER: | 2002:57398 USPATFULL |
| TITLE: | Cosmetic compositions containing resveratrol and retinoids |
| INVENTOR(S): | **Pillai, Sreekumar**, Wayne, NJ, United States<br>Mahajan, Manisha Narayan, Westwood, NJ, United States<br>Granger, Stewart Paton, Paramus, NJ, United States<br>Pocalyko, David Joseph, Wayne, NJ, United States<br>Barratt, Marieann, Oak Ridge, NJ, United States |
| PATENT ASSIGNEE(S): | Unilever Home & Personal Care USA, division of Conopco, Greenwich, CT, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 6358517 | B1 | 20020319 | |
| APPLICATION INFO.: | US 2000-663764 | | 20000918 | (9) |

| | NUMBER | DATE | |
|---|---|---|---|
| PRIORITY INFORMATION: | US 1999-160970P | 19991022 | (60) |

| | |
|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | GRANTED |

crosomes

L12 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2002 ACS

TI Isoenzyme- and species-specific susceptibility of cDNA-expressed **CYP1A** P-450s to different flavonoids

AB The inhibitory and stimulatory effects of 6 flavonoids with distinct hydroxylation patterns on the recombinant and hepatic mouse and human **CYP1A** P 450s were studied. CDNA-expressed mouse CYP1A1 and CYP1A2 differed in their sensitivity to both hydroxylated and nonhydroxylated flavonoids, resp. A comparison between the mouse and human CYP1A2 revealed that .alpha.-naphthoflavone and flavone did not change the benzo[.alpha.]pyrene 3-hydroxylation activity of human CYP1A2 but inhibited its 7-ethoxyresorufin and 7-methoxyresorufin O-dealkylation activities. In contrast, hydroxylated flavonoids increased the 7-methoxyresorufin O-demethylation and acetanilide 4-hydroxylation activities of cDNA-expressed human CYP1A2 and in human liver microsomes. These compds. inhibited the benzo[a]pyrene 3-hydroxylase activity of cDNA-expressed CYP1A1 and CYP1A2s as well as in mouse and human liver microsomes. Hydroxylated flavonoids did not inhibit NADPH-cytochrome P 450 reductase activity but inhibited NADPH-2,6-dichlorophenolindophenol reductase activity in liver microsomes and in microsomes from recombinant Hep G2 cells. Structure-activity relations indicated the importance of OH groups in the 5- and 7-positions on the A ring of the flavane nucleus. These OH groups accounted for the inhibitory potency of chrysin on each of the activities of the expressed P 450s, whereas the presence of a OH group at the 4'-position on the B ring decreased the inhibitory potency of naringenin compared to that of chrysin. The ortho-orientation of a OH group on the B ring was of importance, inasmuch as **quercetin** was more potent than morin as an inhibitor of cDNA-expressed and hepatic microsomal monooxygenases.

ST **cytochrome P450 1A** expression liver flavonoid

IT 117-39-5, **Quercetin** 480-16-0, Morin 480-40-0, Chrysin 480-41-1, Naringenin 525-82-6, Flavone 604-59-1, .alpha.-Naphthoflavone
RL: BIOL (Biological study)
(cytochrome P 450 1A expression by mouse and human liver response to)

TI Isoenzyme- and species-specific susceptibility of cDNA-expressed **CYP1A** P-450s to different flavonoids

1994

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS

AB Sixteen naturally occurring flavonoids were investigated as substrates for cytochrome P 450 in uninduced and Aroclor 1254-induced rat liver microsomes. Naringenin, hesperetin, chrysin, apigenin, tangeretin, kaempferol, galangin and tamarixetin were all metabolized extensively by induced rat liver microsomes but only to a minor extent by uninduced microsomes. No metabolites were detected from eriodictyol, taxifolin, luteolin, **quercetin**, myricetin, fisetin, morin or isorhamnetin. The identity of the metabolites was elucidated using Ic-ms and 1H-NMR, and was consistent with a general metabolic pathway leading to the corresponding 3',4'-dihydroxylated flavonoids either by hydroxylation or demethylation. Structural requirements for microsomal hydroxylation appeared to be a single or no hydroxy group on the B-ring of the flavan nucleus. The presence of two or more hydroxy groups on the B-ring seemed to prevent further hydroxylation. The results indicate that demethylation only occurs in the B-ring when the methoxy group is positioned at C4' and not at the C3,-position. The **CYP1A** isoenzymes were found to be the main enzymes involved in flavonoid hydroxylation, whereas other cytochrome P 450 isoenzymes seem to be involved in flavonoid demethylation.

IT 117-39-5, **Quercetin** 480-16-0, Morin 480-18-2, Taxifolin 480-19-3, Isorhamnetin 480-40-0, Chrysin 480-41-1, Naringenin 481-53-8, Tangeretin 491-70-3, Luteolin 520-18-3, Kaempferol 520-33-2, Hesperetin 520-36-5, Apigenin 528-48-3, Fisetin 529-44-2, Myricetin 548-83-4, Galangin 552-58-9, Eriodictyol 603-61-2, Tamarixetin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(in vitro biotransformation of flavonoids by rat liver microsomes)

IT 9035-51-2, Cytochrome P 450, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(isoenzymes **CYP1A**, CYP3A, CYP1A2, and CYP2B; in vitro biotransformation of flavonoids by rat liver microsomes)

TI In vitro biotransformation of flavonoids by rat liver microsomes

L12 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2002 ACS

TI Inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metabolism by the isoflavonoids **genistein** and equol

AB The inhibitory effect of the isoflavonoids **genistein** and equol on cytochrome P 450 activities has been investigated. **Genistein** and equol inhibited the high capacity component of p-nitrophenol (CYP2E1 substrate) metab. in liver microsomes from acetone-induced mice with IC50 values of approx. 10 mM and 560 .mu.M, resp. (cf. diethyldithiocarbamate, IC50, 69 .mu.M). Using human CYP2E1 from a specific expression system (which overcame multienzyme involvement in the rodent system), non-competitive inhibition was also seen with both isoflavonoids. **Genistein** and equol also inhibited the high capacity component of ethoxyresorufin (**CYP1A** substrate) metab. in liver microsomes from .beta.-naphthoflavone-induced mice with IC50 values of 5.6 mM and 1.7 mM, resp. (cf. .alpha.-naphthoflavone, IC50 0.8.mu.M). Using human CYP1A2 from a specific expression system, noncompetitive inhibition was seen with both isoflavonoids. CYP1A1 inhibition offers a possible explanation for the chemopreventive effect of **genistein** against, for example, dimethylbenz[a]anthracene genotoxicity reported in animals but the IC50 values negate the relevance of this specific chemopreventive action at the levels likely to be achieved from the human diet.

ST chemoprevention genotoxicity isoflavonoid; **genistein** genotoxicity chemoprevention; equol genotoxicity chemoprevention; metab inhibition cell line **genistein** equol

IT Animal cell line
(**CYP 1A** and 2E1; inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT Genotoxicity
Metabolism, animal
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT Isoflavonoids
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT Microsome
(liver; inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT 9035-51-2, Cytochrome P 450, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT 446-72-0, **Genistein** 531-95-3, Equol
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

IT 100-02-7, p-Nitrophenol, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

TI Inhibition of mouse and human **CYP 1A**-and 2E1-dependent

substrate metabolism by the isoflavonoids **genistein** and equol

L12 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
TI Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the **cytochrome P450** **1A** family
IT 117-39-5, **Quercetin** 153-18-4, Rutin 154-23-4, Catechin 446-72-0, **Genistein** 480-16-0, Morin 480-19-3, Isorhamnetin 480-40-0, Chrysin 480-41-1, Naringenin 486-66-8, Daidzein 487-26-3, Flavanone 490-46-0, Epicatechin 491-67-8, Baicalein 491-70-3, Luteolin 520-18-3, Kaempferol 520-33-2, Hesperetin 520-36-5, Apigenin 522-12-3, Quercitrin 525-82-6, Flavone 528-48-3, Fisetin 529-44-2, Myricetin 548-83-4, Galangin 552-58-9, Eriodictyol 552-66-9, Daidzin 577-85-5, Flavonol 578-74-5, Apigetrin 855-97-0 863-03-6, Epicatechin gallate 970-74-1, Epigallocatechin 1061-93-4 1064-06-8 1247-97-8 3681-99-0, Puerarin 9035-51-2, Cytochrome P450, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)
TI Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the **cytochrome P450** **1A** family

ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS

AB A series of homoisoflavonoids and chalcones, isolated from the endemic tropical plant Dracaena cinnabari Balf. (Agavaceae), were tested for their potential to inhibit cytochrome P 4501A (**CYP1A**) enzymes and Fe-enhanced in vitro peroxidn. of microsomal lipids in C57B1/6 mouse liver. The effects of the polyphenolic compds. were compared with those of prototypal flavonoid modulators of **CYP1A** and the well-known antioxidant, butylated hydroxytoluene. 2-Hydroxychalcone and partly 4,6-dihydroxychalcone were found to be strong inhibitors of **CYP1A** -dependent 7-ethoxyresorufin O-deethylase (EROD) activity in vitro comparable to the effects of **quercetin** and chrysin. The first screening of flavonoids and chalcones of Dracaena cinnabari for antioxidant activity was done in an in vitro microsomal peroxidn. assay. While chalcones were shown to be poor antioxidants, 7,8-methylenedioxy-3(4-hydroxybenzyl) chromane, as one of the tested homoisoflavonoids, exhibited a strong antioxidant activity comparable to that of the strongest flavonol antioxidant, **quercetin**.

IT 94-41-7D, chalcone, derivs. 117-39-5, **Quercetin** 480-40-0, Chrysin 548-83-4, Galangin 644-78-0, 2-Hydroxychalcone 6665-86-7, 7-Hydroxyflavone 25515-43-9 148461-99-8 148462-00-4 361160-32-9 361160-34-1 361160-37-4 361160-39-6
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT 332859-78-6, cytochrome **CYP1A**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

TI Chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari: modulations of drug-metabolizing enzymes and antioxidant activity

L12 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS

TI Inhibition of benzo[a]pyrene-induced cytotoxicity and **cytochrome P450 1A** activity by dietary flavonoids in human liver cell model: structure-activity relationship

AB Inhibition of benzo[a]pyrene (B[a]P)-induced cytotoxicity and cytochrome P 450 1A (**CYP 1A**) activity by flavonoids (1-100 .mu.M) was examd. in terms of the structure-activity relationship in the human liver-derived cell model (HepG2). Two hydroxyl groups in the 5- and 7-position of flavonoids were essential to inhibit B[a]P-induced cytotoxicity. Generally, flavones (IC50; 5.0-17.2 .mu.M) were more potent than the corresponding flavonols (IC50; 42.7-131.8 .mu.M), and flavonoids such as apigenin (IC50; 7.2 .mu.M) were more active than the corresponding isoflavonoids, **genistein** (IC50; 61.7 .mu.M). The planar structure of flavone proved to be important in inhibiting B[a]P-induced toxicity and **CYP 1A** activity. The inhibitory effect of flavonoids on B[a]P-induced **CYP 1A** activity was correlated well with the inhibition of B[a]P-induced cytotoxicity ($r = 0.635$, $p < 0.01$).

IT 117-39-5, **Quercetin** 153-18-4, Rutin 154-23-4, Catechin 446-72-0, **Genistein** 480-18-2, Taxifolin 480-19-3, Isorhamnetin 480-41-1, Naringenin 480-44-4, Acacetin 486-66-8, Daidzein 491-70-3, Luteolin 520-18-3, Kaempferol 520-27-4, Diosmin 520-36-5, Apigenin 525-82-6, Flavone 970-74-1, Epigallo catechin 989-51-5, Epigallo catechin gallate 10236-47-2, Naringin
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(inhibition of benzopyrene-induced cytotoxicity and cytochrome P 450 1A

activity by dietary flavonoids in human liver cell model)

TI Inhibition of benzo[a]pyrene-induced cytotoxicity and **cytochrome P450 1A** activity by dietary flavonoids in human liver cell model: structure-acti

L12 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2002 ACS

AB The authors examd. the effects of several agents, including dietary flavonoids, on CYP1A1 expression utilizing a recently developed high-throughput screening system for assessing human cytochrome P 450 (CYP) induction. HepG2 cells, stably integrated with regulatory regions of human CYP1A1, were treated with resveratrol, apigenin, curcumin, kaempferol, green tea ext. (GTE), (-)-epigallocatechin gallate (EGCG), **quercetin**, and naringenin. Of these flavonoids, resveratrol produced the greatest increase in CYP1A1-mediated luciferase activity (10-fold), whereas GTE, apigenin, curcumin, and kaempferol produced 2- to 3-fold increases in activity. Compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), omeprazole, or benzanthracene, where increases in luciferase activity ranged from 12- to 35-fold, these flavonoids exhibited weak agonist activity. The remaining compds., EGCG, **quercetin**, and naringenin, produced negligible effects. Cotreatment of cells with TCDD and GTE, naringenin, and apigenin resulted in 58, 77, and 74% redns., resp., in TCDD-mediated CYP1A1 induction, indicating that these flavonoids exhibit potential antagonist activity toward the aryl hydrocarbon (Ah) receptor. Furthermore, results also suggest that GTE and apigenin possess Ah receptor antagonist and weak agonist activities. Thus, the authors showed that a 96-well plate assay allowing high-throughput screening for P 450 induction in less than 24 h was efficient in detg. the effects of flavonoids on human **CYP1A** expression. Signal-to-noise ratios were low, and well-to-well and replicate variability was below 10%, allowing induction to be easily detected in this system. These features illustrate the reliability and feasibility of this high-vol. screening system for identifying CYP inducers. Furthermore, results produced with the stable cell line were corroborated in HepG2 cells and primary cultures of human hepatocytes, suggesting that stably integrated cell lines harboring enhancer elements of P 450 genes may be highly conducive to high-throughput screening.

IT 117-39-5, **Quercetin** 458-37-7, Curcumin 480-41-1, Naringenin 501-36-0, Resveratrol 520-18-3, Kaempferol 520-36-5, Apigenin 989-51-5, (-)-Epigallocatechin gallate
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)

TI The use of a high-volume screening procedure to assess the effects of dietary flavonoids on human CYP1A1 expression

L12 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS

TI Induction of rat liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides, nitrofurans, and **quercetin**

AB The genotoxic activity of environmental xenobiotics is manifested either in their direct interaction with cellular genetic material or in provoking secondary events, among which reactive oxygen species (ROS) prodn. is a common phenomenon. Both pathways can be mediated by the activity of the cytochrome P 450 monooxygenase system. The authors studied the induction of the **CYP 1A** or CYP 2B monooxygenases in rat liver by the fungicides: thiram, captan, captafol, and dodine and the drugs: nitrofurazone and furazolidone and the plant flavonoid: **quercetin**. A cytochrome P 450 induction assay (CYPIA test) was used. S9 prepd. from the livers of rats treated with the test compds. were used to activate ethidium bromide (EtBr) (**CYP 1A** isoenzyme) or cyclophosphamide (CPA) (CYP 2B isoenzyme) in the Ames test. It was found that among the tested compds., the most potent inducer of **CYP 1A** was furazolidone (3.times. 80 mg/kg). Less potent was thiram (1.times. 100 mg/kg), as well as **quercetin** (3.times. 80 mg/kg), and captafol (1.times. 30 mg/kg). On the other hand, thiram (1.times. 100 mg/kg), captafol (1.times. 30 mg/kg), and **quercetin** (3.times. 80 mg/kg) were most potent in the CYP 2B isoenzyme induction, while furazolidone (3.times. 80 mg/kg), and nitrofurazone (3.times. 80 mg/kg) appeared to be less potent in this respect. Captan and dodine (3.times. 80 mg/kg) did not affect the activity of any of the cytochrome P 450 isoenzymes.

ST liver cytochrome P 450 isoenzyme induction fungicide nitrofuran **quercetin**

IT Fungicides
Genotoxicity
Liver
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)

IT 50-18-0, Cyclophosphamide 1239-45-8, Ethidium bromide
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)

IT 332859-78-6, Cytochrome P 450 1A 334677-51-9, Cytochrome P 450 2B
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)

IT 59-87-0, Nitrofurazone 67-45-8, Furazolidone 117-39-5, **Quercetin** 133-06-2, Captan 137-26-8, Thiram 2425-06-1, Captafol 2439-10-3, Dodine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)

TI Induction of rat liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides, nitrofurans, and **quercetin**

L12 ANSWER 1 OF 14 USPATFULL

| | |
|---|---|
| ACCESSION NUMBER: | 2002:301080 USPATFULL |
| TITLE: | Compositions and methods for induction of proteins involved in xenobiotic metabolism |
| INVENTOR(S): | Raucy, Judy, San Diego, CA, UNITED STATES |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2002168623 | A1 | 20021114 |
| APPLICATION INFO.: | US 2001-832621 | A1 | 20010411 (9) |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 2000-196681P | 20000412 (60) |
| | US 2000-241391P | 20001017 (60) |

| | |
|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | APPLICATION |
| LEGAL REPRESENTATIVE: | DAVID R PRESTON & ASSOCIATES, 12625 HIGH BLUFF DRIVE, SUITE 205, SAN DIEGO, CA, 92130 |
| NUMBER OF CLAIMS: | 20 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 24 Drawing Page(s) |
| LINE COUNT: | 2077 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 2 OF 14 USPATFULL

| | |
|---|---|
| ACCESSION NUMBER: | 2002:174964 USPATFULL |
| TITLE: | Use of fluorescein aryl ethers in high throughput cytochrome P450 inhibition assays |
| INVENTOR(S): | Miller, Vaughn P., Arlington, MA, United States<br>Streser, David, Natick, MA, United States<br>Crespi, Charles L., Marblehead, MA, United States |
| PATENT ASSIGNEE(S): | Gentest Corporation, Woburn, MA, United States (U.S. corporation) |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 6420131 | B1 | 20020716 |
| APPLICATION INFO.: | US 2000-636332 | | 20000810 (9) |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 1999-149762P | 19990819 (60) |
| | US 1999-150044P | 19990820 (60) |

| | |
|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | GRANTED |
| PRIMARY EXAMINER: | Lilling, Herbert J. |
| LEGAL REPRESENTATIVE: | Wolf, Greenfield & Sacks, P.C. |
| NUMBER OF CLAIMS: | 5 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 10 Drawing Figure(s); 5 Drawing Page(s) |
| LINE COUNT: | 1019 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS

| | |
|---|---|
| ACCESSION NUMBER: | 2001:772033 CAPLUS |
| DOCUMENT NUMBER: | 135:314787 |
| TITLE: | Induction of rat liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides, nitrofurans, and **quercetin** |
| AUTHOR(S): | Rahden-Staron, Iwonna; Czeczot, Hanna; Szumilo, Maria |
| CORPORATE SOURCE: | Department of Biochemistry, Medical University of Warsaw, Warsaw, 02-097, Pol. |

SOURCE: Mutation Research (2001), 498(1-2), 57-66
CODEN: MUREAV; ISSN: 0027-5107
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:628000 CAPLUS
DOCUMENT NUMBER: 135:343870
TITLE: Effect of onion consumption by rats on hepatic drug-metabolizing enzymes
AUTHOR(S): Teyssier, C.; Amiot, M.-J.; Mondy, N.; Auger, J.; Kahane, R.; Siess, M.-H.
CORPORATE SOURCE: UMR de Toxicologie Alimentaire, INRA-Universite de Bourgogne, Dijon, 21065, Fr.
SOURCE: Food and Chemical Toxicology (2001), 39(10), 981-987
CODEN: FCTOD7; ISSN: 0278-6915
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:544261 CAPLUS
DOCUMENT NUMBER: 135:241436
TITLE: The use of a high-volume screening procedure to assess the effects of dietary flavonoids on human CYP1A1 expression
AUTHOR(S): Allen, Scott W.; Mueller, Lisa; Williams, Susanne N.; Quattrochi, Linda C.; Raucy, Judy
CORPORATE SOURCE: Puracyp, LLC, San Diego, CA, USA
SOURCE: Drug Metabolism and Disposition (2001), 29(8), 1074-1079
CODEN: DMDSAI; ISSN: 0090-9556
PUBLISHER: American Society for Pharmacology and Experimental Therapeutics
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:300371 CAPLUS
DOCUMENT NUMBER: 135:235878
TITLE: Chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari: modulations of drug-metabolizing enzymes and antioxidant activity
AUTHOR(S): Machala, Miroslav; Kubinova, Renata; Horavova, Pavla; Suchy, Vaclav
CORPORATE SOURCE: Veterinary Research Institute, Brno, 62132, Czech Rep.
SOURCE: Phytotherapy Research (2001), 15(2), 114-118
CODEN: PHYREH; ISSN: 0951-418X
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:57732 CAPLUS
DOCUMENT NUMBER: 134:189342

TITLE: Inhibition of benzo[a]pyrene-induced cytotoxicity and **cytochrome P450 1A** activity by dietary flavonoids in human liver cell model: structure-activity relationship
AUTHOR(S): Kim, Hyun-Jung; Chun, Hyang-Sook; Yang, Ryung
CORPORATE SOURCE: Food Chemistry and Biotechnology Division, Korea Food Research Institute, Sungnam, 463-420, S. Korea
SOURCE: Biotechnology Letters (2000), 22(24), 1941-1946
CODEN: BILED3; ISSN: 0141-5492
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:548843 CAPLUS
DOCUMENT NUMBER: 131:295057
TITLE: Modulation of murine phenobarbital-inducible CYP2A5, CYP2B10 and **CYP1A** enzymes by inhibitors of protein kinases and phosphatases
AUTHOR(S): Posti, Katja; Leinonen, Susanna; Tetri, Sami; Kottari, Sami; Viitala, Pirkko; Pelkonen, Olavi; Raunio, Hannu
CORPORATE SOURCE: Department of Pharmacology and Toxicology, University of Oulu, Oulu, FIN-90220, Finland
SOURCE: European Journal of Biochemistry (1999), 264(1), 19-26
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:409182 CAPLUS
DOCUMENT NUMBER: 129:157870
TITLE: Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the **cytochrome P450 1A** family
AUTHOR(S): Kanazawa, Kazuki; Yamashita, Takatoshi; Ashida, Hitoshi; Danno, Gen-Ichi
CORPORATE SOURCE: Department of Biofunctional Chemistry, Faculty of Agriculture, Kobe University, Kobe, 657-8501, Japan
SOURCE: Bioscience, Biotechnology, and Biochemistry (1998), 62(5), 970-977
CODEN: BBBIEJ; ISSN: 0916-8451
PUBLISHER: Japan Society for Bioscience, Biotechnology, and Agrochemistry
DOCUMENT TYPE: Journal
LANGUAGE: English

L12 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:404741 CAPLUS
DOCUMENT NUMBER: 129:94932
TITLE: Inhibition of mouse and human **CYP 1A** -and 2E1-dependent substrate metabolism by the isoflavonoids **genistein** and equol
AUTHOR(S): Helsby, N. A.; Chipman, J. K.; Gescher, A.; Kerr, D.
CORPORATE SOURCE: School of Biochemistry, University of Birmingham, Edgbaston, B15 2TT, UK
SOURCE: Food and Chemical Toxicology (1998), 36(5), 375-382
CODEN: FCTOD7; ISSN: 0278-6915
PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal
LANGUAGE: English

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:302020 CAPLUS
DOCUMENT NUMBER: 129:49167
TITLE: In vitro biotransformation of flavonoids by rat liver microsomes
AUTHOR(S): Nielsen, S. E.; Breinholt, V.; Justesen, U.; Cornett, C.; Dragsted, L. O.; Nielsen, S. E.; Breinholt, V.; Justesen, U.; Cornett, C.; Dragsted, L. O.
CORPORATE SOURCE: Institute of Toxicology and t Institute of Food Chemistry and Nutrition, Danish Veterinary and Food Administration, Soborg, DK-2860, Den.
SOURCE: Xenobiotica (1998), 28(4), 389-401
CODEN: XENOBH; ISSN: 0049-8254
PUBLISHER: Taylor & Francis Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:424712 CAPLUS
DOCUMENT NUMBER: 125:80284
TITLE: Specificity of substrate and inhibitor probes for cytochrome P450s: evaluation of in vitro metabolism using cDNA-expressed human P450s and human liver microsomes
AUTHOR(S): Ono, S.; Hatanaka, T.; Hotta, H.; Satoh, T.; Gonzalez, F. J.; Tsutsui, M.
CORPORATE SOURCE: Amersham K.K., Central Lab. for Research and Development, Chiba, 270-14, Japan
SOURCE: Xenobiotica (1996), 26(7), 681-693
CODEN: XENOBH; ISSN: 0049-8254
PUBLISHER: Taylor & Francis
DOCUMENT TYPE: Journal
LANGUAGE: English

L12 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:528697 CAPLUS
DOCUMENT NUMBER: 121:128697
TITLE: Isoenzyme- and species-specific susceptibility of cDNA-expressed **CYP1A** P-450s to different flavonoids
AUTHOR(S): Tsyrlov, Ilya B.; Mikhailenko, Victor M.; Gelboin, Harry V.
CORPORATE SOURCE: Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Biochimica et Biophysica Acta (1994), 1205(2), 325-35
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

L12 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:250399 CAPLUS
DOCUMENT NUMBER: 118:250399
TITLE: Differential inhibition of coumarin 7-hydroxylase activity in mouse and human liver microsomes
AUTHOR(S): Maenpaa, Jukka; Sigusch, Holger; Raunio, Hannu; Syngelma, Tuula; Vuorela, Pia; Vuorela, Heikki; Pelkonen, Olavi
CORPORATE SOURCE: Dep. Pharmacol. Toxicol., Univ. Oulu, Oulu, SF-90220,

Finland
SOURCE: Biochemical Pharmacology (1993), 45(5), 1035-42
CODEN: BCPCA6; ISSN: 0006-2952
DOCUMENT TYPE: Journal
LANGUAGE: English

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Generate Collection

L1: Entry 8 of 25 File: USPT Jan 15, 2002

DOCUMENT-IDENTIFIER: US 6338855 B1
TITLE: Cleansing articles for skin and/or hair which also deposit skin care actives

Brief Summary Text (126):
Anti-wrinkle, anti-skin atrophy and skin repair actives can be effective in replenishing or rejuvenating the epidermal layer. These actives generally provide these desirable skin care benefits by promoting or maintaining the natural process of desquamation. Nonlimiting examples of antiwrinkle and anti-skin atrophy actives include retinoic acid and its derivatives (e.g., cis and trans); retinal; retinol; retinyl esters such as retinyl acetate, retinyl palmitate, and retinyl propionate; vitamin B.sub.3 compounds (such as niacinamide and nicotinic acid), salicylic acid and derivatives thereof (such as 5-octanoyl salicylic acid, heptyloxy 4 salicylic acid, and 4-methoxy salicylic acid); sulfur-containing D and L amino acids and their derivatives and salts, particularly the N-acetyl derivatives, a preferred example of which is N-acetyl-L-cysteine; thiols, e.g. ethane thiol; hydroxy acids, phytic acid, lipoic acid; lysophosphatidic acid; skin peel agents (e.g., phenol and the like); Actein 27-Deoxyactein Cimicifugoside (available from Cirnigoside); adapalene; ademethionine; adenosine; aletris extract; alkyl glutathione esters; alkoxyalkoxy alkoxyn benzoic and derivatives; aloe derived lectins; amino propane phosphoric acid; 3-aminopropyl dihydrogen phosphate; Amadorine (available from Barnet Products); anise extracts; AOSINE (available from Secma); arginine amino benzoate; ASC III (available from E. Merck, located in Darmstadt, Germany); ascorbic acid; ascorbyl palmitate; asiatic acid; asiaticosides; ARLAMOL GEO.TM. (available from ICI, located in Wilmington, Del.); azaleic acid; benzoic acid derivatives; bertholletia extracts; betulinic acid; BIOCHANIN A AND BIOPEPTIDE CL (available from Sederma, located in Brooklyn, N.Y.); BIOPEPTIDE EL (available from Sederma); biotin; blackberry bark extract; blackberry lily extracts; black cohosh extract; blue cohesh extract; butanoyl betulinic acid; carboxymethyl 1,3 beta glucan; catecholamnines; chalcones; citric acid esters; chaste tree extract; clover extracts; coumestrol; CPC Peptide (available from Barnet Products); daidzein; dang gui extract; darutoside; debromo laurinterol; 1-decanoyl-glycero-phosphonic acid; dehydrocholesterol; dehydrodicreosol; dehydrodieugenol; dehydroepiandersterone; DERMOLECTINE (available from Sederma); dehydroascorbic acid; dehydroepiandersterone sulfate; dianethole; dihydroxy benzoic acid; 2,4 dihydroxybenzoic acid; diglycol guanidine succinate; diosgenin; disodium ascorbyl phosphate; dodecanedioic acid; Ederline (available from Seporga); Enderline (available from Laboratories Seporga); equol; eriodictyol; estrogen and its derivatives; ETF (available from Laboratories Seporga); ethocyn; ELESERYL SH (available from Laboratories Serobiologiques, located in Somerville, N.J.); ENDONUCLEINE (available from Laboratories Serobiologiques); ergosterol; eythrobic acid; fennel extract; fenugreek seed extract; FIBRASTIL (available from Sederma); FIBROSTIMULINES S and P (available from Sederma); FIRMOGEN LS 8445 (available from Laboratories Serobiologiques); formononetin; forsythia fruit extract; gallic acid esters; gamma amino butyric acid; GATULINE RC (available from Gattlefosse, located in Priest, France); genistein; genisteine; genistic acid; gentisyl alcohol; gingko bilboa extracts; ginseng extracts; ginsenoside (RO, R.sub.6-1, R.sub.6-2, R.sub.6-3, R.sub.C, R.sub.D, R.sub.E, R.sub.F, R.sub.F-2, R.sub.G-1, R.sub.G-2); gluco pyranosyl-L-ascorbate; glutathione and its esters; glycitein; hesperitin; hexahydro curcumin; HMG- coenzyme A reductase inhibitors; hops extracts; 11 hydroxy undecanoic acid; 10 hydroxy decanoic acid; 25-hydroxycholesterol; 7-hydroxylated sterols; hydroxyethyl isostearyloxy isopropanolamine; hydroxy-tetra methyl piperidinyloxy; hypotaurine; ibukijakou extract; isoflavone SG 10 (available from Barnet Products); kinetin; kohki extract; L-2-OXO-thiazolidine-4-carboxylic acid esters; lactate dehydrogenase inhibitors; 1-lauryl, -lyso-phosphatidyl choline; lectins; lichochalcone LF15 (available from Maruzen); licorice extracts; lignan; lumisterol; lupenes; luteolin; lysophosphitidic acid; magnesium ascorbyl phosphate; margin; melatonin; melibiose; metalloproteinase

Generate Collection

L5: Entry 2 of 43 File: USPT Oct 15, 2002

DOCUMENT-IDENTIFIER: US 6465709 B1
TITLE: Exothermic bandage

Detailed Description Text (16):
Examples of retinoids include but not limited to retinol (Vitamin A alcohol), retinal (Vitamin A aldehyde), retinyl acetate, etinyl palmitate, retinoic cid, 9-cis-retinoic acid and 13-cis-retinoic acid. Examples of flavonoids include but not limited to naringenin, quercetin, catechins (e.g., epigallocatechin gallate), theaflavins, robustaflavone, hinokiflavone, amentoflavone, agathisflavone, volkensiflavone, morelloflavone, rhusflavanone, and succedangeaflavanone.

inhibitors; methoprene; methoprenic acid; mevalonic acid; MPC COMPLEX (available from CLR); N methyl serine; N methyl taurine; N, N.sup.1 -bis (lactyl) cysteamine; naringenin; neotigogenin; o-desmethylangoiensin; oat beta glucan; oleanolic acid; pantethine; phenylalanine; photoanethone; piperdine; placental extracts; pratensein; pregnenolone; pregnenolone acetate; pregnenolone succinate; premarin; quillaic acid; raloxifene; REPAIR FACTOR 1 and REPAIR FACTOR FCP (both available from Sederna); retinoates (esters of C.sub.2 -C.sub.20 alcohols); retinyl glucuronate; retinyl linoleate; S-carboxymethyl cysteine; SEANAMINE FP (available from Laboratories Serobiologiques); sodium ascorbyl phosphate; soya extracts; spleen extracts; tachysterol; taurine; tazarotene; tempol; thymulen; thymus extracts; thyroid hormones; tigogenin; tocopheryl retinoate; toxifolin; traumatic acid; tricholine citrate; trifoside; uracil derivatives; ursolic acid; vitamin D.sub.3 and its analogs; vitamin K; vitex extract; yam extract; yamogenin; zeatin; and mixtures thereof.

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L1: Entry 16 of 25 File: USPT Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146658 A
TITLE: Prodrugs, their preparation and use as pharmaceuticals

Brief Summary Text (24):
drug is a compound which is linked via a hydroxyl, amino or imino group and has a biological effect, preferably a pharmaceutical agent, particularly preferably an anthracycline which is linked via a hydroxyl or, when p=0, non-3'-amino group, preferably doxorubicin, 4'-epidoxorubicin, 4- or 4'-deoxydoxorubicin or a compound preferably selected from the group comprising etoposides, N,N-bis(2-chloroethyl)-hydroxyaniline, 4-hydroxycyclophosphamide, vindesine, vinblastine, vincristine, terfenadine, terbutaline, fenoterol, salbutamol, muscarine, oxyphenbutazone, salicylic acid, paminosalicylic acid, 5-fluorouracil, 5-fluorocytidine, 5-fluorouridine, methotrexate, diclofenac, flufenamic acid, 4-methylaminophenazone, theophylline, nifedipine, mitomycin C, mitoxantrone, camptothecin, m-AMSA, taxol, nocodazole, colchicine, cyclophosphamide, rachelmycin, cisplatin, melphalan, bleomycin, nitrogen mustard, phosphoramide mustard, quercetin, genistein, erbstatin, tyrphostin, rohitukin derivative ((-)-cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-1-benzopyran-4-one; EP 89119710.5), retinoic acid, butyric acid, phorbol ester, DMSO, aclacinomycin, progesterone, buserelin, tamoxifen, mifepristone, onapristone, N-(4-aminobutyl)-5-chloro-2-naphthalenesulfonamide, pyridinyloxazol-2-one, quinolyl-, isoquinolyloxazol-2-one, staurosporine, ethanolamine, verapamil, forskolin, 1,9-dideoxyforskolin, quinine, quinidine, reserpine, methyl 18-O-(3,5-dimethoxy-4-hydroxybenzoyl)reserpate, lonidamine, buthionine-sulfoximine, diethyl dithiocarbamate, cyclosporin A, azathioprine, chlorambucil, N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide (WO 91/17748). 15-deoxyspergualine, FK 506, ibuprofen, indomethacin, aspirin, sulfasalazine, penicillamine, chloroquine, dexamethasone, prednisolone, lidocaine, propafenone, procaine, mefenamic acid, paracetamol, 4-aminophenazone, muskosine, orciprenaline, isoprenaline, amiloride, p-nitrophenyl guanidinobenzoate or their derivatives additionally substituted by one or more hydroxyl, amino or imino groups.

Brief Summary Text (41):
compound in which the glycosyl radical can be cleaved off by enzymatic hydrolysis, in which the spacer can be spontaneously cleaved off by chemical hydrolysis, in which the drug is a pharmaceutical agent or one of its derivatives obtained by introducing additional hydroxyl, amino or imino groups, which is more hydrophilic than the drug, which leads in vivo to fewer toxic reactions than the drug itself, in which the drug is a pharmacologically active substance, in which the drug is additionally substituted by one or more hydroxyl, amino or imino groups and slows down tumor growth, in which the drug is a standard cytostatic, in which the drug is an antimetabolite, in which the drug is 5-fluorouracil, 5-fluorocytidine, 5-fluorouridine, cytosine arabinoside or methotrexate, in which the drug is a substance which intercalates into DNA, in which the drug is doxorubicin, daunomycin, idarubicin, epirubicin or mitoxantrone, in which the drug inhibits topoisomerase I+II, in which the drug is camptothecin, etoposide or M-AMSA, in which the drug is a tubulin inhibitor, in which the drug is vincristine, vinblastine, vindesine, taxol, nocodazole, colchicine or etoposide, in which the drug is an alkylating agent, in which the drug is cyclophosphamide, mitomycin C, rachelmycin, cisplatin, phosphoramide mustard, melphalan, bleomycin, nitrogen mustard or N,N-bis(2-chloroethyl)-4-hydroxyaniline, in which the drug is neocarcinostatin, calicheamicin, dynemicin or esperamicin A, in which the drug is a compound which inactivates ribosomes, in which the drug is verrucarin A, in which the drug is a tyrosine phosphokinase inhibitor, in which the drug is quercetin, genistein, erbstatin, tyrphostin or rohitukin derivative, in which the drug is a

differentiation inducer, in which the drug is retinoic acid, butyric acid, phorbol ester, DMSO or aclacinomycin, in which the drug is a hormone, hormone agonist or hormone antagonist, in which the drug is progesterone, buserelin, tamoxifen, mifepristone or onapristone, in which the drug is a substance which alters the pleiotropic resistance to cytostatics, in which the drug is a calmodulin inhibitor, in which the drug is a protein kinase C inhibitor, in which the drug is a P-glycoprotein inhibitor, in which the drug is a modulator of mitochondrially bound hexokinase, in which the drug is an inhibitor of b-glutamylcysteine synthetase or of glutathione Stransferase, in which the drug is an inhibitor of superoxide dismutase, in which the drug is an inhibitor of the proliferation-associated protein defined by MAb Ki67 in the cell nucleus of cells-undergoing division, in which the drug is a substance which has immunosuppressant effects, in which the drug is a standard immunosuppressant, in which the drug is a macrolide, in which the drug is cyclosporine A, rapamycin, FK 506, in which the drug is azathioprine, methotrexate, cyclophosphamide or chorambual, in which the drug is a substance which has an anuinflammatory effect, in which the drug is a non-steroidal anuinflammatory substance, in which the drug is a slow-acting antirheumatic drug, in which the drug is a steroid, in which the drug is a substance which has antiinflammatory, analgesic or antipyretic effect, in which the drug is a derivative of an organic acid, in which the drug is a non-acidic analgesic/antiinflammatory agent, in which the drug is oxyphenbutazone, in which the drug is a local anesthetic, in which the drug is an antiarrhythmic, in which the drug is a Ca++ antagonist, in which the drug is an antihistaminic, in which the drug is an inhibitor of phosphodiesterase, in which the drug is a parasympathomimetic, in which the drug is a sympathomimetic or in which the drug is a substance with an inhibitory effect on human urokinase; and moreover compound in which the

**End of Result Set**

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L1: Entry 25 of 25 File: USPT Jun 4, 1996

DOCUMENT-IDENTIFIER: US 5523087 A
TITLE: Pharmaceutical compositions for the treatment of diabetic male sexual dysfunction

Detailed Description Paragraph Table (1):

| Constituents | Chemicals | Active Compound |
|---|---|---|
| Soybean | Genistein, | 45 mg phytoestrogens |

Daidzein, (calculated as and their glycosides free aglycon form) Lecithin Phosphatidyl choline 200 mg from soybean Phytosterol Beta-sitosterol 10 mg from soybean (Sitosteryl-D-glucoside) Damiama leaf Damiana leaf dry extract 30 mg Vitamin A Retinol palmitate 1 mg Vitamin B1 Thiamine mononitrate 50 mg Vitamin B6 Pyridoxine hydrochloride 50 mg Vitamin E alpha-Tocopheryl 30 mg acetate or succinate Calcium Calcium carbonate 150 mg Magnesium Magnesium oxide 300 mg Zinc Zinc sulfate 80 mg

Detailed Description Paragraph Table (2):

| Constituents | Chemicals | Active Compound |
|---|---|---|
| Soybean | Genistein, | 60 mg phytoestrogens |

Daidzein, (calculated as and their glycosides free aglycon form) Lecithin Phosphatidyl choline 200 mg from soybean Phytosterol Beta-sitosterol 50 mg from soybean (Sitosteryl-D-glucoside) Damiama leaf Damiana leaf dry extract 100 mg Vitamin A Retinol palmitate 3 mg Vitamin B1 Thiamine mononitrate 50 mg Vitamin B6 Pyridoxine hydrochloride 150 mg Vitamin E alpha-Tocopheryl 30 mg acetate or succinate Calcium Calcium carbonate 150 mg Magnesium Magnesium oxide 500 mg Zinc Zinc sulfate 80 mg

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**Search Results** - Record(s) 1 through 10 of 25 returned.

1. 6444221 . 12 Oct 99; 03 Sep 02. Methods of treating chronic inflammatory diseases using carbonyl trapping agents. Shapiro; Howard K.. 424/451; 424/439 424/442 424/457 424/464 424/468 514/458 514/55 514/57. A61K009/48.

2. 6441027 . 17 May 99; 27 Aug 02. Method of regulating the female reproductive system through angiogenesis inhibitors. D'Amato; Robert J., et al. 514/450;. A61K031/335.

3. 6416758 . 28 Apr 00; 09 Jul 02. Antibody conjugate kits for selectively inhibiting VEGF. Thorpe; Philip E., et al. 424/145.1; 424/1.49 424/1.53 424/1.69 424/133.1 424/134.1 424/135.1 424/141.1 424/142.1 424/178.1 424/179.1 424/181.1 424/183.1 424/195.11 424/9.2 424/9.3 435/69.1 435/69.6 435/69.7 435/7.23 435/70.21 435/810 530/387.3 530/388.1 530/388.15 530/388 .24 530/391.3 530/391.7 530/391.9. A61K038/36 C12P021/08 C07K016/22.

4. 6406693 . 12 Jul 99; 18 Jun 02. Cancer treatment methods using antibodies to aminophospholipids. Thorpe; Philip E., et al. 424/130.1; 424/132.1 424/133.1 424/135.1 424/138.1 424/141.1 424/152.1 424/184.1 435/6 530/387.1. A61K039/395 C07K016/00 C07K016/28 C07K016/30 C12Q001/68.

5. 6399655 . 22 Dec 98; 04 Jun 02. Method for the prophylactic treatment of cataracts. de Juan, Jr.; Eugene. 514/456; 514/912. A61K031/35.

6. 6342221 . 28 Apr 00; 29 Jan 02. Antibody conjugate compositions for selectively inhibiting VEGF. Thorpe; Philip E., et al. 424/178.1; 424/1.49 424/1.53 424/130.1 424/179.1 424/181.1 424/183.1 424/193.1 424/195.11 424/9.3 424/9.34 424/9.6 435/69.1 435/7.1 435/7.21 435/7.23 435/70.21 435/810 530/391.1 530/391.3 530/391.5 530/391.7 530/391.9. A61K039/44 A61K039/395.

7. 6342219 . 28 Apr 00; 29 Jan 02. Antibody compositions for selectively inhibiting VEGF. Thorpe; Philip E., et al. 424/145.1; 424/133.1 424/134.1 424/135.1 424/141.1 424/142.1 424/143.1 435/335 435/69.1 435/810 530/387.1 530/387.3 530/388.1 530/388.15 530/388.23 530/391.1 530/391.3 530/391.5 530/391.7 530/809 530/864 530/865 530/866. A61K039/395 C12P021/08 C07K016/00.

8. 6338855 . 22 Apr 99; 15 Jan 02. Cleansing articles for skin and/or hair which also deposit skin care actives. Albacarys; Lourdes Dessus, et al. 424/409; 424/402. A01N025/34 A01N025/08.

9. 6330472 . 29 Jan 99; 11 Dec 01. Prophylactic and therapeutic treatment of the ductal epithelium for a mammary gland for cancer. Sukumar; Saraswati Vaidyanathan. 604/21; 514/1 514/2. A61N001/30 A61K031/00.

10. 6316465 . 28 Jun 99; 13 Nov 01. Ophthalmic uses of PPARgamma agonists and PPARgamma antagonists. Pershadsingh; Harrihar A., et al. 514/310; 514/912 514/914. A61K031/41.

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| (L1 SAME L2).USPT. | 25 |

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**Search Results** - Record(s) 11 through 20 of 25 returned.

11. 6312694. 12 Jul 99; 06 Nov 01. Cancer treatment methods using therapeutic conjugates that bind to aminophospholipids. Thorpe; Philip E., et al. 424/178.1; 424/133.1 424/134.1 424/135.1 424/136.1 424/137.1 424/141.1 424/142.1 424/143.1 424/181.1 424/193.1 514/12 530/387.1 530/388.1. A61K039/395 C12P021/08 C07K016/00.

12. 6287602. 15 Sep 99; 11 Sep 01. Treatment of oncologic tumors with an injectable formulation of a Golgi apparatus disturbing agent. Singh; Saira Sayed. 424/488; 424/485 514/449. A61K009/14.

13. 6190678. 04 Sep 98; 20 Feb 01. Cleansing and conditioning products for skin or hair with improved deposition of conditioning ingredients. Hasenoehrl; Erik John, et al. 424/401; 424/402 424/443 424/446. A61K007/00 A61K007/50 A61K013/00.

14. 6153208. 11 Sep 98; 28 Nov 00. Cleansing and conditioning article for skin or hair. McAtee; David Michael, et al. 424/402; 424/401 424/404 424/443 424/59 424/70.19 424/70.21 424/70.22 424/70.31 424/70.8 424/709 510/130 510/135 510/136 510/137. A01N025/34 A61K007/42 A61K007/06 A61K007/075 A61K009/70.

15. 6153184. 08 Jun 98; 28 Nov 00. Destruction of the epithelium of an exocrine gland in the prophylactic and therapeutic treatment of cancer. Sukumar; Saraswati Vaidyanathan. 424/93.6; 424/93.1 424/93.2 435/455 514/2 514/44. A01N063/00.

16. 6146658. 20 May 97; 14 Nov 00. Prodrugs, their preparation and use as pharmaceuticals. Bosslet; Klaus, et al. 424/450; 514/23 514/25 514/53 514/54 514/8. A23B004/03 A61K031/70 A61K038/16.

17. 6028099. 13 Mar 98; 22 Feb 00. Use of an inhibitor of the protein tyrosine kinase pathway in the treatment of choroidal neovascularization. de Juan, Jr.; Eugene. 514/434; 514/456 514/912. A67K031/35 A67K031/389.

18. 6017949. 01 Aug 97; 25 Jan 00. Method of regulating the female reproductive system through angiogenesis inhibitors. D'Amato; Robert J., et al. 514/450;. A61K031/335.

19. 5980929. 13 Mar 98; 09 Nov 99. Use of a protein tyrosine kinase pathway inhibitor in the treatment of retinal ischmemia or ocular inflammation. de Juan, Jr.; Eugene. 424/427; 424/423. A61F002/14 A61F002/02.

20. 5955100. 24 May 95; 21 Sep 99. Prodrugs their preparation and use as pharmaceuticals. Bosslet; Klaus, et al. 424/450; 514/2 514/23 514/25 514/34 514/8 536/1.11 536/18.1 536/4.1 536/6.4. A61K009/127 A61K031/70 C07H015/00.

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**Search Results** - Record(s) 21 through 25 of 25 returned.

21. 5919813 . 13 Mar 98; 06 Jul 99. Use of a protein tyrosine kinase pathway inhibitor in the treatment of diabetic retinopathy. de Juan, Jr.; Eugene. 514/432; 514/451 514/453 514/456 514/866. A61K031/38 A61K031/35.

22. 5902792 . 22 May 97; 11 May 99. Method of inducing apoptosis in cancer cells. Jayaram; Hiremagalur N.. 514/23; 436/64. A01N043/04.

23. 5763415 . 02 Aug 96; 09 Jun 98. Destruction of the epithelium of an exocrine gland in the prophylactic and therapeutic treatment of cancer. Sukumar; Saraswati Vaidyanathan. 514/44; 424/93.1 424/93.21 435/235.1 435/325 435/69.1 514/2. A61K048/00.

24. 5621002 . 09 Sep 94; 15 Apr 97. Prodrugs for enzyme mediated activation. Bosslet; Klaus, et al. 514/451; 435/188.5 514/461. A61K031/335 A61K031/34.

25. 5523087 . 15 Feb 95; 04 Jun 96. Pharmaceutical compositions for the treatment of diabetic male sexual dysfunction. Shlyankevich; Mark. 424/757; 424/725 424/774 514/182 514/456. A61K035/78 A61K031/56 A61K031/35.

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|---|---|
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| (L1 SAME L2).USPT. | 25 |

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Cancer Research, Vol 50, Issue 11 3367-3376, Copyright © 1990 by American Association for Cancer Research



ARTICLES

# Metabolism of 2-acetylaminofluorene and benzo(a)pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450

**ME McManus, WM Burgess, ME Veronese, A Huggett, LC Quattrochi and RH Tukey**
Department of Clinical Pharmacology, School of Medicine, Flinders University of South Australia, Bedford Park.

The human P-450 CYP1A1 gene and a P450IA2 complementary DNA have been expressed in Cos-1 cells and the expressed proteins were assayed for their capacity to metabolize the carcinogens 2-acetylaminofluorene (AAF), benzo(a)pyrene, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was determined. The expressed human P450IA1 and P450IA2 proteins, when run on a 7.5% sodium dodecyl sulfate-polyacrylamide gel, migrated with different mobilities, with the former displaying the lower molecular weight. In human liver microsomes from 18 subjects, only a protein band corresponding to P450IA2 was detectable. Cos-1 cell-expressed P450IA1 and P450IA2 were capable of N-hydroxylating AAF and these activities were inhibited by alpha-naphthoflavone. In human liver microsomes, a correlation of r = 0.76 (P less than 0.05; n = 18) was obtained between AAF N-hydroxylase activity and P450IA2 content. AAF N-hydroxylase activity of human liver microsomes was also strongly inhibited by alpha-naphthoflavone. Except in the case of PhIP, where both proteins exhibited similar activities, P450IA2 was at least an order of magnitude more efficient than P450IA1 in activating IQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, and 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline to mutagens as measured in the Ames test. Statistically significant correlations were obtained between IQ activation and P450IA2 content (r = 0.75, r2 = 0.56) and PhIP activation and P450IA2 content (r = 0.71, r2 = 0.5) in human liver microsomes. The activation of both IQ and PhIP by expressed proteins and human liver microsomes was strongly inhibited by alpha-naphthoflavone. The above data suggest a

major role for P450IA2 in activation (N-hydroxylation) of aromatic amides and amines in human liver. When benzo(a)pyrene hydroxylase activity was determined, only Cos-1 cell-expressed P450IA1 exhibited appreciable activity. While alpha-naphthoflavone inhibited Cos-1 cell-expressed P450IA1 benzo(a)pyrene hydroxylase activity, it caused a marked stimulation of this activity in human liver microsomes, which lack P450IA1 protein. The lack of a role for P450IA proteins in benzo(a)pyrene metabolism is further supported by the poor correlation (r = 0.43, P greater than 0.05) between this activity and P450IA2 content of human liver microsomes. However, when P450IIIA3 content of the above human liver microsomes was determined by using the Western blot technique and correlated with benzo(a)pyrene metabolism, an r value of 0.70 (P less than 0.5) was obtained. These data suggest that human P450IIIA proteins are involved in benzo(a)pyrene metabolism.

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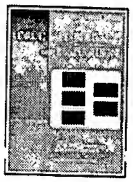

DRUG METABOLISM AND DISPOSITION ▸HOME

T. Shimada, Y. Oda, E. M. J. Gillam, F. P. Guengerich, and K. Inoue
**Metabolic Activation of Polycyclic Aromatic Hydrocarbons and Other Procarcinogens by Cytochromes P450 1A1 and P450 1B1 Allelic Variants and Other Human Cytochromes P450 in Salmonella typhimurium NM2009**
Drug Metab. Dispos., September 1, 2001; 29(9): 1176 - 1182.
[Abstract] [Full Text] [PDF]

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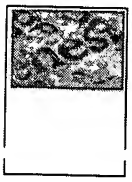

Carcinogenesis ▸HOME

M.-F. Yueh, N. Nguyen, M. Famourzadeh, C. P. Strassburg, Y. Oda, F.P. Guengerich, and R. H. Tukey
**The contribution of UDP-glucuronosyltransferase 1A9 on CYP1A2-mediated genotoxicity by aromatic and heterocyclic amines**
Carcinogenesis, June 1, 2001; 22(6): 943 - 950.
[Abstract] [Full Text] [PDF]

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Cancer Research ▸HOME

E. M. M. van Lieshout, H. M. J. Roelofs, S. Dekker, C. J. J. Mulder, T. Wobbes, J. B. M. J. Jansen, and W. H. M. Peters
**Polymorphic Expression of the Glutathione Polymorphic Expression of the Glutathione S-Transferase P1 Gene and Its Susceptibility to Barrett's Esophagus and Esophageal Carcinoma**
Cancer Res., February 1, 1999; 59(3): 586 - 589.
[Abstract] [Full Text] [PDF]

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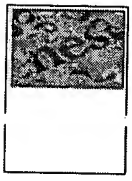

Carcinogenesis ▸HOME

N. Song, W. Tan, D. Xing, and D. Lin
**CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China**
Carcinogenesis, January 1, 2001; 22(1): 11 - 16.
[Abstract] [Full Text]

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Carcinogenesis ▸HOME

C. Agus, K. F. Ilett, F. F. Kadlubar, and R. F. Minchin
**Characterization of an ATP-dependent pathway of activation for the heterocyclic amine carcinogen N-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline**
Carcinogenesis, June 1, 2000; 21(6): 1213 - 1219.
[Abstract] [Full Text]

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Molecular Human Reproduction ▸HOME

P. L.M. Zusterzeel, W. L.D.M. Nelen, H. M.J. Roelofs, W. H.M. Peters, H. J. Blom, and E. A.P. Steegers

**Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss**

Mol. Hum. Reprod., May 1, 2000; 6(5): 474 - 478.

[Abstract] [Full Text]

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TOXICOLOGICAL SCIENCES ▸HOME

K. F. Windmill, A. Gaedigk, P. de la M. Hall, H. Samaratunga, D. M. Grant, and M. E. McManus

**Localization of N-Acetyltransferases NAT1 and NAT2 in Human Tissues**

Toxicol. Sci., March 1, 2000; 54(1): 19 - 29.

[Abstract] [Full Text]

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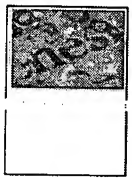

Carcinogenesis ▸HOME

F. P. Guengerich

**Metabolism of chemical carcinogens**

Carcinogenesis, March 1, 2000; 21(3): 345 - 351.

[Abstract] [Full Text]

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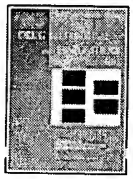

DRUG METABOLISM AND DISPOSITION ▸HOME

M. Nakajima, S. Nakamura, S. Tokudome, N. Shimada, H. Yamazaki, and T. Yokoi

**Azelastine N-Demethylation by Cytochrome P-450 (CYP)3A4, CYP2D6, and CYP1A2 in Human Liver Microsomes: Evaluation of Approach to Predict the Contribution of Multiple CYPs**

Drug Metab. Dispos., December 1, 1999; 27(12): 1381 - 1391.

[Abstract] [Full Text]

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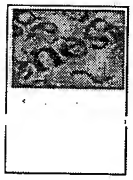

Carcinogenesis ▸HOME

H. A.J. Schut and E. G. Snyderwine

**DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis**

Carcinogenesis, March 1, 1999; 20(3): 353 - 368.

[Abstract] [Full Text]

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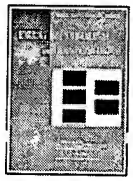

DRUG METABOLISM AND DISPOSITION ▸HOME

E. T. Morgan, M. B. Sewer, H. Iber, F. J. Gonzalez, Y.-H. Lee, R. H. Tukey, S. Okino, T. Vu, Y.-H. Chen, J. S. Sidhu, and C. J. Omiecinski

**Physiological and Pathophysiological Regulation of Cytochrome P450**

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[Abstract] [Full Text]

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# Involvement of Human CYP1A Isoenzymes in the Metabolism and Drug Interactions of Riluzole *In Vitro*[1]

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## Abstract



Cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) isoenzymes involved in riluzole oxidation and glucuronidation were characterized in (1) kinetic studies with human hepatic microsomes and isoenzyme-selective probes and (2) metabolic studies with genetically expressed human CYP isoenzymes from transfected B-lymphoblastoid and yeast cells. *In vitro* incubation of [$^{14}$C]riluzole (15 μM) with human hepatic microsomes and NADPH or UDPGA cofactors resulted in formation of N-hydroxyriluzole ($K_m$ = 30 μM) or an unidentified glucuroconjugate ($K_m$ = 118 μM). Human microsomal riluzole N-hydroxylation was most strongly inhibited by the CYP1A2 inhibitor α-naphthoflavone ($IC_{50}$ = 0.42 μM). Human CYP1A2-expressing yeast microsomes generated N-hydroxyriluzole, whereas human CYP1A1-expressing yeast microsomes generated N-hydroxyriluzole, two additional hydroxylated derivatives and an O-dealkylated derivative. CYP1A2 was the only genetically expressed human P450 isoenzyme in B-lymphoblastoid microsomes to metabolize riluzole. Riluzole glucuronidation was inhibited most potently by propofol, a substrate for the human hepatic UGT HP4 (UGT1.8/9) isoenzyme. *In vitro*, human hepatic microsomal hydroxylation of riluzole (15 μM) was weakly inhibited by amitriptyline, diclofenac, diazepam, nicergoline, clomipramine, imipramine, quinine and enoxacin ($IC_{50} \approx$ 200-500 μM) and cimetidine ($IC_{50}$ = 940 μM). Riluzole (1 and 10 μM) produced a weak, concentration-dependent inhibition of CYP1A2 activity and showed competitive inhibition of methoxyresorufin O-demethylase. Thus, riluzole is predominantly metabolized by CYP1A2 in human hepatic microsomes to N-hydroxyriluzole;

extrahepatic CYP1A1 can also be responsible for the formation of several other metabolites. Direct glucuronidation is a relatively minor metabolic route. *In vivo*, riluzole is unlikely to exhibit significant pharmacokinetic drug interaction with coadministered drugs that undergo phase I metabolism.

# Introduction

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Riluzole[1] [2-amino-6-(trifluoromethoxy)benzothiazole], a novel antiglutamate agent with neuroprotective properties in animal models of neurodegenerative disease (Doble, 1996), has been shown to prolong survival in patients with ALS (Bensimon *et al.*, 1994; Lacomblez *et al.*, 1996). After oral administration to humans, the drug is almost completely absorbed, undergoes limited first-pass metabolism and is excreted predominantly *via* the urine in the form of metabolites resulting from phase I and II metabolism.[2,3]

Characterization of the CYP isoenzymes responsible for the metabolism of riluzole is of importance in assessing the likelihood of pharmacokinetic variability due to genetic polymorphism and differential regulation and in identifying potential drug interactions. In the present study, the *in vitro* oxidative metabolism and glucuronidation of riluzole were investigated using human hepatic microsomes. Identity of the CYP isoenzymes involved in riluzole biotransformation was established using genetically expressed human CYP isoenzymes from transfected cell lines and yeast and isoenzyme-selective inhibitory probes. Similarly, pathways of hepatic microsomal glucuronidation of riluzole were investigated with known inhibitors/substrates of UGT isoenzymes. To identify potential metabolic drug interactions, the effects of known CYP substrates/inhibitors and frequently coadministered drugs on the hepatic microsomal oxidation of riluzole and, conversely, the effects of riluzole on specific human hepatic CYP-dependent drug metabolism reactions were determined.

# Materials and Methods

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## Chemicals

Riluzole, N-hydroxyriluzole (RPR 112512), 4-hydroxyriluzole (RP 65077), 5-hydroxyriluzole (RP 65110), 7-hydroxyriluzole (RP 65331) and 2-amino-6-hydroxybenzothiazole (RPR 109792) were synthesized at the Centre de Recherche de Vitry/Alfortville, Rhône-Poulenc Rorer (France) and the Collegeville Chemical Processing Center, Rhône-Poulenc Rorer (Collegeville, PA). [$^{14}$C]Riluzole (radiochemical purity, >99%, specific activity, 56 mCi/mmol) was synthesized by the Service of Labeled Molecules of the Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). Aspirin, captopril, diazepam, enoxacin, imipramine, metronidazole, nicergoline, paracetamol, pefloxacin, ranitidine and sparfloxacin were obtained from Rhône-Poulenc Rorer. Baclofen, amitriptyline, amoxicillin, chlorpropamide, chlorzoxazone, cimetidine, clomipramine, coumarin, methoxyresorufin, nifedipine, resorufin, theophylline, thiamine, tolbutamide, troleandomycin, aniline, isoniazid, α-naphthoflavone, reduced NADPH, UDPGA, sulfaphenazole, Brij-58, 1-naphthol, 4-methylumbelliferone, lithocholic

acid, bilirubin, androsterone and β-estradiol were purchased from Sigma Chemical Co. (St. Louis, MO). Furafylline and S-mephenytoin were purchased from Ultrafine Chemicals (Manchester, UK); acetanilide and quinidine sulfate were obtained from E. Merck (Darmstadt, Germany); caffeine and quinine sulfate were from Prolabo (Paris, France); SR-mephenytoin was from Sandoz (Basel, Switzerland); ketoconazole was from Biomol Research Laboratories (Plymouth Meeting, PA) and bufuralol was from Gentest Corp. (Woburn, MA). All other reagents were purchased from commercial sources and were of analytical grade.

## Biological Materials

**Human liver samples and preparation of microsomes.** Human liver samples were obtained from male and female organ transplant donors (Eurotransplant) or from surgery (Hôpital Cochin, Paris, France). Microsomal fractions were prepared by differential ultracentrifugation. After tissue homogenization in 20 mM Tris·HCl buffer, pH 7.4, containing 0.15 M KCl, the microsomal fraction was isolated from the supernatant of a 20-min 9000 × *g* spin by ultracentrifugation at 105,000 × *g* for 60 min. The microsomal precipitate was suspended in 100 mM potassium phosphate buffer, pH 7.4, and recentrifuged at 105,000 × *g* for an additional 60 min. The final precipitate was resuspended in the phosphate buffer and stored at −80°C until required. A pool of human liver microsomes was also obtained from Human Biologics Inc.

Microsomes from human B-lymphoblastoid cell lines genetically engineered to express human CYP isoenzymes CYP1A1 (batch M102B), CYP1A2 (M103C), CYP2D6 (M105b), CYP2E1 (M106i) and CYP3A4 (M107d) were obtained from Gentest Corp.

Yeast cells (*Saccharomyces cerevisiae*) genetically engineered to express human CYP isoenzymes (CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18, CYP3A4 and CYP3A5) and to overexpress yeast CYP450 reductase were obtained from CNRS (Gif-sur-Yvette, France) and INSERM (Paris, France), and microsomes were prepared from these cells at Rhône-Poulenc Rorer (Vitry-sur-Seine, France) as part of the Bioavenir Program. Microsomes from the same yeast strain but not expressing human CYP, were used as controls.

## Microsomal Incubation

**Riluzole biotransformation.** Riluzole oxidation and glucuronidation were assayed by microsomal incubation of [$^{14}$C]-radiolabeled and unlabeled drug in the presence of the respective cofactors NADPH and UDPGA. For oxidative reactions, incubations with hepatic microsomes were performed with a suspension of hepatic microsomes (protein content, 2 mg/ml) in potassium phosphate buffer (0.1 M, pH 7.4) containing NADPH (1 mM) and $MgCl_2$ (10 mM). Lymphoblast B-cell microsomes were incubated in the same medium at a 0.5 mg/ml protein content, according to the supplier's instructions, resulting in final enzyme concentrations of 8, 18, 105, 38 and 17 pmol/ml for CYP1A1, CYP1A2, CYP2D6, CYP2E1 and CYP3A4, respectively. Yeast microsomes were incubated in Tris·HCl buffer (50 mM, pH 7.4) containing EDTA (1 mM) and NADPH (1 mM) in the absence of cytochrome $b_5$. For yeast microsome incubations, the final CYP450 content was 200 pmol/ml. For glucuronidation reactions, [$^{14}$C]riluzole (15 μM) was incubated with a suspension of hepatic microsomes (protein content,

1 mg/ml) in potassium phosphate buffer (0.1 M, pH 7.4) containing UDPGA (5 mM) and $MgCl_2$ (5 mM). For glucuronidation reactions, microsomes were activated with an optimal concentration (0.2 mg/mg of protein) of Brij-58 detergent. Incubations were performed at 25°C (yeast microsomes) or 37°C (hepatic and B-cell microsomes) in an agitating water bath, and reactions were initiated by the addition of NADPH (oxidative metabolism) or UDPGA (glucuronidation). Riluzole incubation mixtures were sampled until 20 min for monooxygenase-catalyzed reactions (30 min for expressed enzymes) and until 60 min for glucuronosyltransferase-catalyzed reactions. Reactions were terminated by the addition of an equivalent volume of methanol/acetonitrile (3.6:1 v/v) to the incubation mixture. The resulting mixture was then centrifuged at 30,000 × *g* for 10 min, and the supernatant was stored at 4°C before analysis.

**Enzyme kinetics.** Enzyme kinetic studies were performed by incubation of [$^{14}$C]riluzole at concentrations of 2 to 1000 μM with hepatic and CYP1A2-expressing yeast cell microsomes.

**Interaction studies.** In inhibition studies, hepatic microsomes were preincubated for 10 min with varying concentrations of CYP isoenzyme substrates/inhibitors (1-1000 μM) or UGT inhibitors (1-100 μM) in the presence of the appropriate cofactor (NADPH or UDPGA) before the addition of [$^{14}$C]riluzole (15 μM). Specific CYP isoenzyme probes included α-naphthoflavone, acetanilide and caffeine for CYP1A (Birkett *et al.*, 1993; Gonzalez, 1992), tolbutamide and sulfaphenazole for CYP2C8/9 (Birkett *et al.*, 1993), omeprazole and mephenytoin for CYP2C19 (Andersson *et al.*, 1993; Birkett *et al.*, 1993), quinidine for CYP2D6, with quinine as a negative control (Birkett *et al.*, 1993; Gonzalez, 1992), aniline, *p*-nitrophenol, chlorzoxazone and isoniazid for CYP2E1 (Birkett *et al.*, 1993; Zand *et al.*, 1993) and ketoconazole and troleandomycin for CYP3A (Back *et al.*, 1989; Birkett *et al.*, 1993). UGT inhibitors included propofol, 1-naphthol, 4-methylumbelliferone, lithocholic acid, bilirubin, androsterone, estradiol and *p*-nitrophenol.

**Metabolic drug/drug interactions.** In drug interaction studies with riluzole used as the substrate, hepatic microsomes were preincubated for 10 min with clomipramine, diclofenac, amitriptyline, imipramine, enoxacin, quinine, theophylline, cimetidine, caffeine, ranitidine, paracetamol, pyridoxine, enalapril, thiamine, captopril, pefloxacin, aspirin, amoxicillin, metronidazole, piracetam, baclofen, nicergoline or diazepam at concentrations ranging from 2 to 1000 μM in the presence of NADPH before the addition of [$^{14}$C]riluzole (15 μM).

Incubations were carried out in triplicate for experiments at single inhibitor concentrations; for determination of $IC_{50}$ values, single incubations were performed at multiple inhibitor concentrations. $K_i$ determinations were performed in duplicate.

**Effects of riluzole on marker enzyme activities.** In an additional series of drug interaction studies, the inhibitory effects of riluzole were determined on the following CYP-selective oxidative reactions: nifedipine dehydrogenation (a marker for CYP3A4) (Guengerich *et al.*, 1986); chlorzoxazone-6-hydroxylation (a marker for CYP2E1) (Peter *et al.*, 1991); bufuralol-1-hydroxylation (a marker for CYP2D6) (Kronbach *et al.*, 1987); S-mephenytoin-4-hydroxylation (a marker for CYP2C19) (Meier *et al.*, 1985; Wrighton *et al.*, 1993); tolbutamide-4-hydroxylation (a marker for

CYP2C9) (Knodell *et al.*, 1987; Veronese *et al.*, 1991); coumarin-7-hydroxylation (a marker for CYP2A6) (Pearce *et al.*, 1992; Yun *et al.*, 1991); and phenacetin O-deethylation (a marker for CYP1A2) (Distlerath *et al.*, 1985; Sattler *et al.*, 1992). Human hepatic microsomes were incubated with riluzole (1 and 10 μM), and the various enzyme substrates at concentrations approximating or in excess of published $K_m$ values. Parallel experiments were conducted with two human liver samples, and for each assay, analyses were performed in duplicate or triplicate with a NADPH- or glucose-6-phosphate-free control to quantify nonenzymatic drug metabolism. All incubation mixtures were analyzed by high-performance liquid chromatography.

The kinetic methoxyresorufin O-demethylation assay was performed on 96-well microplates in 200 μl of potassium phosphate buffer (75 mM, pH 7.64), 9 mM KCl and 1.4 mM NADH at 37°C with 0.2 mg/ml human liver microsomes or 6.6 pmol/ml yeast-expressed CYP1A2. Methoxyresorufin dissolved in DMSO and riluzole dissolved in methanol were added together to the incubation mixture, and the reaction was initiated by the addition of 500 μM NADPH (final concentration). The final incubation mixture contained 0.5% (v/v) of both solvents. Methoxyresorufin concentrations were 0.1, 0.3, 0.5, 2 and 5 μM. For each methoxyresorufin concentration, riluzole concentrations of 0, 3, 15, 30, 150 and 300 μM were tested. Resorufin production was monitored continuously with fluorescence detection (excitation wavelength, 544 nm; emission wavelength, 590 nm) over 10 min using a LabSystems Fluoroscan II microplate spectrofluorometer controlled by Biolise software. Concentrations were calculated from a resorufin standard curve.

**Sample Analysis**

Analysis of riluzole metabolites was carried out by high-performance liquid chromatography with a Kontron 360 automatic sampler, a 420 solvent delivery pump, a Kontron 430 UV detector (265 nm) and a Berthold LB507A radiodetector equipped with a 500-μl flow cell. The system was controlled by a Kontron MT2 Datasystem. Separation was achieved on a Lichrocart 125 × 4-mm column with a Lichrocart 4 × 4-mm guard column, both packed with Lichrosphere 60 RP Select B 5-μm particles (Merck Clevenot). The mobile phase consisted of 10 mM $K_2HPO_4$/methanol/acetonitrile/glacial acetic acid (108:72:20:1 v/v/v/v), eluting at a flow rate of 1 ml/min. The flow rate of the scintillation fluid was 3 ml/min, and the efficiency of the radiodetector cell was 77%.

Standard riluzole samples were prepared in phosphate buffer and mixed with methanol/acetonitrile as for the incubation samples. UV detection of riluzole and its metabolites was linear over the concentration range of 1 to 1000 μM. The radiodetector, which was calibrated by comparing [$^{14}$C]riluzole peak areas with radioactivity counts in a Beckman LS 6000SC liquid scintillation counter, yielded linear detection over a concentration range of 1.35 to 500 μM.

**Data Analysis**

The kinetic parameters of riluzole metabolism [$V_{max}$, apparent $K_m$, $K_i$ and $IC_{50}$ (defined as the inhibitor concentration reducing riluzole hydroxylation by 50%)] were calculated by iterative nonlinear regression analysis using GraFit Version 3.0 software. Intrinsic metabolic clearance ($Cl_m$) was calculated as

$V_{max}/K_m$. Results are expressed as mean with S.E.M.

# Results

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## Riluzole Monooxidation

**Enzyme kinetics.** [$^{14}$C]Riluzole was metabolized in an NADPH-dependent (monooxygenase-catalyzed) manner on incubation with human hepatic microsomes, resulting in the formation of the N-hydroxylated derivative. The mean rate of riluzole N-hydroxylation by hepatic microsomes from 6 individuals was 138 ± 53 pmol/min/mg. The rate increased linearly with microsomal protein concentration up to 2.5 mg/ml. The reaction followed normal single-enzyme Michaelis-Menten kinetics (fig. 1); apparent kinetic parameters $V_{max}$ and $K_m$ for metabolite formation are shown in table 1.

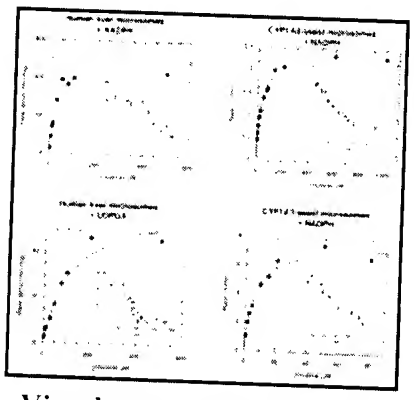

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**Fig. 1.** Enzyme kinetics of riluzole N-hydroxylation and glucuronidation by human hepatic microsomes and by yeast microsomes expressing human CYP1A isoenzymes. Top left, saturation curve for riluzole hydroxylation by human hepatic microsomes. Top right, saturation curve for riluzole hydroxylation by yeast microsomes expressing human CYP1A2. Bottom right, saturation curve for riluzole hydroxylation by yeast microsomes expressing human CYP1A1. Bottom left, saturation curve for riluzole glucuronidation by human hepatic microsomes. Insets, Eadie-Hofstee transformations of the same data: saturation functions were obtained by fitting untransformed data to a simple Michaelis-Menten function using iterative nonlinear regression analysis.

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TABLE 1
**Michaelis-Menten kinetic parameters for riluzole biotransformation by human hepatic microsomes and CYP1A-expressing yeast microsomes**

**Isoenzymes involved in phase I biotransformation of riluzole.** A number of isoenzyme-selective substrates and inhibitors were screened for their ability to inhibit N-hydroxylation of riluzole by hepatic microsomes (table 2). Riluzole N-hydroxylation was markedly reduced by pretreatment with the CYP1A inhibitor α-naphthoflavone (80% inhibition at 1 μM). Consecutive experiments over a range of concentrations showed that α-naphthoflavone inhibited riluzole N-hydroxylation with an $IC_{50}$ value of 0.42 μM. The CYP1A2 substrates caffeine (37% inhibition at 1 mM) and acetanilide (21% inhibition at 1 mM) produced a less-marked inhibition. The CYP2E1 inhibitor chlorzoxazone also weakly inhibited

riluzole N-hydroxylation (36% inhibition at 100 μM; $IC_{50}$ = 287 μM), but other inhibitors of this isoenzyme, such as aniline, isoniazid and *p*-nitrophenol, had minimal effect. Some inhibition of riluzole N-hydroxylation was observed with the CYP2C19 substrate omeprazole (31% inhibition at 100 μM) and with the CYP2D6 substrate quinidine (20% inhibition at 5 μM) but also with the negative control, quinine (table 2). Tolbutamide (a CYP2C8/9 substrate), sulfaphenazole (a CYP2C9 inhibitor), mephenytoin (a CYP2C19 substrate), ketoconazole and troleandomycin (CYP3A4 inhibitors) had no appreciable effect on riluzole N-hydroxylation.

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TABLE 2
**Inhibition of hepatic microsomal oxidation of riluzole by specific substrates/inhibitors of cytochrome P450 isoenzymes**

On incubation of [$^{14}$C]riluzole with NADPH and microsomes from human cytochrome P450-expressing B-lymphoblastoid cells, N-hydroxylation was confined to those containing CYP1A2, with no metabolism occurring with CYP1A1-, CYP2D6-, CYP2E1- or CYP3A4-containing microsomes or control microsomes. In the case of human cytochrome P450-expressing yeast cells, microsomes containing CYP1A2 generated N-hydroxyriluzole on incubation with [$^{14}$C]riluzole, whereas microsomes containing CYP1A1 gave rise to the hydroxylated derivatives N-hydroxyriluzole, 4-hydroxyriluzole and 5-hydroxyriluzole; the O-dealkylated derivative 2-amino-6-hydroxybenzothiazole; and, to a lesser extent, 7-hydroxyriluzole (fig. 2). Maximum riluzole biotransformation rates with CYP1A2- and CYP1A1-containing yeast microsomes were 2.24 and 7.61 pmol/min/pmol of P450, with a $K_m$ value of 25.7 and 6.2 μM, respectively. Yeast microsomes containing CYP3A4, CYP3A5, CYP2C8, CYP2C9 or CYP2C18 and control microsomes did not produce any detectable metabolite.

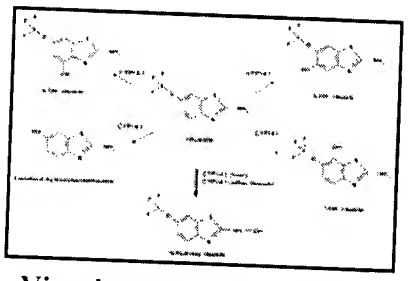

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**Fig. 2.** Metabolic pathways involved in phase I biotransformation of riluzole.

**Effects of drugs on riluzole biotransformation.** Of the drugs screened for their effect on riluzole N-hydroxylation by human hepatic microsomes, the most potent inhibitors were amitriptyline, clomipramine, diazepam, diclofenac, and nicergoline, with $IC_{50}$ values of 210 to 260 μM (table 3). Enoxacin, imipramine and quinine (all at 1 mM) also caused >50% inhibition of riluzole

N-hydroxylation. Intermediate inhibition (25-50%) was observed with theophylline, cimetidine, caffeine, ranitidine and paracetamol, whereas minimal inhibition (<10%) was seen with thiamine, captopril, pefloxacin, aspirin, amoxicillin, metronidazole, piracetam and baclofen at concentrations of 1 mM. Sparfloxacin (≥400 μM) had no effect on parent riluzole biotransformation but did reduce levels of N-hydroxyriluzole, suggesting that it may react directly with this metabolite rather than with the parent compound.

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TABLE 3
**Effects of coadministered drugs on *in vitro* N-hydroxylation of riluzole by human hepatic microsomes**

**Effect of riluzole on P450 enzyme activities.** Riluzole at 1 and 10 μM had a weak inhibitory effect on human hepatic microsomal CYP1A2-mediated phenacetin O-deethylation, CYP2A6-mediated coumarin-7-hydroxylation, CYP2D6-mediated bufuralol-1-hydroxylation and CYP2E1-mediated chlorzoxazone-6-hydroxylation (table 4). Only the effects on phenacetin O-deethylation and chlorzoxazone-6-hydroxylation were concentration dependent. Because riluzole is metabolized by the CYP1A2 isoenzyme, we studied the inhibition kinetics of CYP1A2-catalyzed methoxyresorufin-O-demethylation. Riluzole competitively inhibited methoxyresorufin-O-demethylation with an inhibition constant ($K_i$) of 12.1 ± 1.5 μM in human liver microsomes and 16.7 ± 1.4 μM in microsomes from CYP1A2-expressing yeast (fig. 3). No appreciable or consistent inhibition of CYP2C9-catalyzed tolbutamide 4-hydroxylation, CYP2C19-catalyzed S-mephenytoin 4-hydroxylation or CYP3A4-catalyzed nifedipine dehydrogenation was seen with riluzole.

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TABLE 4
**Effects of riluzole (1 and 10 μM) on human hepatic cytochrome P450-mediated oxidative drug metabolism**

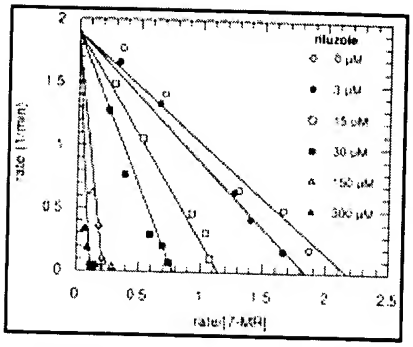


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**Fig. 3.** Inhibition by riluzole of methoxyresorufin O-demethylation by yeast microsomes expressing human CYP1A2. Data are presented as Eadie-Hofstee transformations of saturation curves obtained in the presence of various concentrations of riluzole; saturation functions were obtained by fitting untransformed data to a simple Michaelis-Menten function using iterative nonlinear regression analysis.

### Riluzole Glucuronidation

**Enzyme kinetics.** Riluzole was metabolised in an UDPGA-dependent (*i.e.*, UGT catalyzed) manner on incubation with detergent-activated human hepatic microsomes, resulting in the formation of a single unidentified metabolite. The reaction followed normal single-enzyme Michaelis-Menten kinetics (fig. 1); apparent Michaelis-Menten kinetic parameters $V_{max}$ and $K_m$ are shown in table 1.

### Inhibition of riluzole glucuronidation

Riluzole glucuronidation was inhibited in a concentration-dependent manner by preincubation with propofol ($IC_{50}$ = 18.7 μM), indicating the involvement of the UGT HP4 (UGT1.8/9) isoenzyme in this reaction (Ebner and Burchell, 1993). Maximum inhibition (70%) was seen with propofol 100 μM, whereas less-marked inhibition was obtained with 100 μM estradiol (40% inhibition), 100 μM androsterone (38% inhibition), 100 μM lithocholic acid (37% inhibition), 50 μM bilirubin (28% inhibition) and 100 μM *p*-nitrophenol (23% inhibition).

## Discussion

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Two types of human hepatic enzyme are involved in the biotransformation of riluzole by the human liver *in vitro*: monooxygenases and UDP-glucuronosyltransferases. Quantitatively, monooxygenation is possibly the more important reaction because intrinsic clearance *via* this route was 30-fold higher than that *via* direct glucuronidation.

Riluzole N-hydroxylation was the only monooxygenase-mediated reaction observed with human hepatic microsomes. CYP1A2 appears to be the main isoenzyme involved in this reaction, a conclusion that is based on the potent inhibition observed with the specific CYP1A2 inhibitor α-naphthoflavone ($IC_{50}$ = 0.42 μM), the specific biotransformation noted with genetically expressed CYP1A2, and the competitive inhibitory effect of riluzole on CYP1A2-catalyzed methoxyresorufin O-demethylation. The relatively weak inhibitory effect of the CYP1A2 substrates caffeine and acetanilide on riluzole

N-hydroxylation may be attributed to the low affinity of these compounds for CYP1A2 ($K_m$ = 0.5 to 1.5 mM) (Grant *et al.*, 1987) compared with that of riluzole ($K_m$ = 23 μM). In keeping with the present finding, the CYP1A2 isoenzyme has previously been shown to catalyze specifically the N-hydroxylation of many heterocyclic (aryl) amines in humans (Boobis *et al.*, 1994; Gonzalez and Idle, 1994). Because CYP1A2 seems to be the only CYP1A isoenzyme expressed in human liver (Gonzalez, 1992), it can be concluded that this is the major isoenzyme involved in the hepatic metabolism of riluzole. CYP1A isoenzymes are readily induced *in vivo* by tobacco smoke (Guengerich and Shimada, 1991). Interestingly, increased riluzole clearance in smokers has been demonstrated in a recent population pharmacokinetic study of riluzole.[4]

Possible extrahepatic metabolism of riluzole is suggested by the finding that microsomes of genetically engineered yeast cells expressing human CYP1A1 catalyzed the formation of several hydroxylated derivatives that have previously been identified in the urine of patients treated with riluzole.[2] The CYP1A1 isoenzyme is expressed largely in extrahepatic tissue, such as the lung (Gonzalez, 1992).

Although the disparate effects of the various CYP2E1 substrates on riluzole N-hydroxylation appear somewhat contradictory (some inhibition occurring with chlorzoxazone, but not with aniline, isoniazid or *p*-nitrophenol), it should be noted that not all these substrates are highly specific for CYP2E1. Thus, although chlorzoxazone and *p*-nitrophenol share similarly high affinities ($K_m \approx 30$ μM) for CYP2E1 (Peter *et al.*, 1991; Tassaneeyakul *et al.*, 1993a), chlorzoxazone is also metabolized by CYP1A2 (Ono *et al.*, 1996). The lack of inhibitory effect of *p*-nitrophenol at concentrations as high as 1 mM and the absence of metabolism by CYP2E1 expressed in B-lymphoblastoid cells suggest that this isoenzyme does not play an appreciable role in riluzole oxidation.

The use of *in vitro* systems such as human hepatic microsomes in drug-interaction studies is recommended for predicting the consequences of concurrent drug therapy (Peck *et al.*, 1993). As a substrate for specific P450 isoforms, riluzole has the potential to act as a competitive enzyme inhibitor and thereby alter the metabolism and pharmacokinetics of coadministered drugs that are also subject to phase I metabolism. Effectively, riluzole is a competitive inhibitor of CYP1A2-catalyzed methoxyresorufin O-demethylation, with a $K_i$ value close to its $K_m$ value. At *in vitro* concentrations of 1 and 10 μM, similar to or higher than those achieved therapeutically,[3] riluzole had a weak inhibitory effect on human hepatic microsomal CYP1A2-, CYP2A6-, CYP2D6- and CYP2E1-mediated oxidative drug metabolism. Apart from inhibition of methoxyresorufin O-demethylation, the most pronounced, concentration-dependent inhibition (28%) was that of CYP2E1-catalyzed chlorzoxazone-6-hydroxylation. However, as mentioned above, this inhibition probably reflects on CYP1A2 as well as CYP2E1. Inhibition by riluzole of microsomal CYP1A2-catalyzed phenacetin O-deethylation is not unexpected given the evidence for the involvement of this isoenzyme in riluzole metabolism. However, the weak inhibitory effect of riluzole suggests that it is unlikely to alter to any appreciable extent the hepatic clearance of drugs that are oxidized by the CYP system.

Not surprisingly, known drug substrates of CYP1A2, including enoxacin (Edwards *et al.*, 1988), cimetidine (Knodell *et al.*, 1991), paracetamol (Raucy *et al.*, 1989), imipramine (Lemoine *et al.*, 1993) and the methylxanthines caffeine and theophylline (Fuhr *et al.*, 1992; Tassaneeyakul *et al.*, 1993b),

had an inhibitory effect ($IC_{50}$ values ≥ 400 μM) on riluzole N-hydroxylation. In contrast to enoxacin, and in keeping with their lack of effect on theophylline metabolism *in vivo* or *in vitro* (Edwards *et al.*, 1988), the quinolones pefloxacin and sparfloxacin had no direct effect on riluzole hydroxylation. The inhibitory effect of cimetidine, a well known CYP inhibitor *in vivo* (Smith and Kendall, 1988), was comparatively weak ($IC_{50}$ = 937 μM). However, it has previously been noted that cimetidine inhibition can be underestimated *in vitro*, possibly because its interaction with CYP proceeds rather slowly (Chang *et al.*, 1992).

Although the enzymes responsible for clomipramine and amitriptyline metabolism have not been identified, both these tricyclic antidepressants are susceptible to interaction with fluvoxamine, a potent CYP1A2 inhibitor (Berchty *et al.*, 1991; Brøsen *et al.*, 1993). Moreover, both imipramine and amitriptyline have been shown to be mechanism-based inhibitors of CYP (Murray and Field, 1992), so an effect of these drugs on riluzole metabolism is not unexpected.

For all the tested drugs, the $IC_{50}$ value was ≥14 times greater than the riluzole concentration (15 μM) in the incubate. Therefore, inhibition of riluzole metabolism appears *a priori* unlikely, but results from *in vivo* drug-interaction studies are required before it can be concluded that these agents effectively inhibit riluzole metabolism or alter its pharmacokinetics in humans.

Knowledge of UGT isoenzymes and their substrate specificity is much more limited than is the case with the CYP system. Nevertheless, using genetically expressed enzymes, Ebner and Burchell (1993) established that within the *UGT1* gene family, propofol is a specific substrate for UGT HP4 (UGT1.8/9), whereas 1-naphthol is more specific for UGT HP1 and bilirubin is specific for UGT HP2 and UGT HP3. Therefore, in view of the pronounced inhibitory effect of propofol and the minimal effect of 1-naphthol and bilirubin, riluzole conjugation is most likely to be mediated by the UGT HP4 isoenzyme. Although several other compounds, many of them substrates for UGT2 isoenzymes (androsterone, estradiol, lithocholic acid), inhibited riluzole glucuronidation to varying extents, lack of substrate specificity and the low biotransformation rate of riluzole make it difficult to evaluate the significance of these findings.

In conclusion, riluzole is predominantly metabolized by CYP1A2 in human hepatic microsomes, whereas extrahepatic CYP1A1 is also responsible for the formation of several human metabolites that are also observed *in vivo*. The fact that riluzole is a specific substrate for the CYP1A2 isoenzyme, has a single oxidative metabolic pathway in the liver and is a nontoxic drug with low metabolic clearance in humans could make it an interesting candidate as an *in vitro* and *in vivo* probe. This is further demonstrated by the effect of tobacco use on riluzole clearance in patients with ALS. Direct glucuronidation is a relatively minor metabolic route and is catalyzed by UGT HP4. On the basis of *in vitro* findings, at therapeutic doses riluzole is unlikely to alter the pharmacokinetics of coadministered drugs that undergo phase I metabolism. Conversely, significant modification of the pharmacokinetics of riluzole by these drugs would not be anticipated in clinical practice, although this has yet to be confirmed.

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## Footnotes

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## Abbreviations

ALS, amyotrophic lateral sclerosis; CYP, cytochrome P450; NADPH, reduced nicotinamide adenine dinucleotide phosphate; UGT, uridine diphosphate glucuronosyltransferase; UDPGA, uridine diphosphate glucuronic acid.

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# In Vitro Inhibition and Induction of Human Hepatic Cytochrome P450 Enzymes by Modafinil

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## Abstract



The ability of modafinil to affect human hepatic cytochrome P450 (CYP) activities was examined in vitro. The potential for inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11 by modafinil (5-250 μM) was evaluated with pooled human liver microsomes. Modafinil exhibited minimal capacity to inhibit any CYP enzyme, except CYP2C19. Modafinil inhibited the 4'-hydroxylation of *S*-mephenytoin, a marker substrate for CYP2C19, reversibly and competitively with a $K_i$ value of 39 μM, which approximates the steady-state $C_{max}$ value of modafinil in human plasma at a dosage of 400 mg/day. No irreversible inhibition of any CYP enzyme was observed, and there was no evidence of metabolism-dependent inhibition. The potential for induction of CYP activity was evaluated by exposing primary cultures of human hepatocytes to modafinil (10-300 μM). Microsomes were then prepared and assayed for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 activities. The mean activities of microsomal CYP1A2, CYP2B6, and CYP3A4/5 from modafinil-treated hepatocytes were higher (up to 2-fold) than those in the solvent-treated controls but were less than those produced by reference inducers of these enzymes. At high concentrations of modafinil (≥100 μM), the mean activity of CYP2C9 was decreased (up to 60%) relative to that in the solvent controls. Overall, modafinil was shown to have effects on human hepatic CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4/5 activities in vitro. Although effects obtained in vitro are not always predictive of effects in vivo, such results provide a rational basis for understanding drug-drug interactions that are observed clinically and for planning subsequent investigations.

# Introduction



Modafinil (*dl*-2-[(diphenylmethyl)sulfinyl]acetamide; Fig. 1), a nonamphetamine-like wakefulness-promoting agent, has recently been approved in the United States, United Kingdom, and Ireland (under the tradename Provigil) for the treatment of excessive daytime sleepiness associated with narcolepsy. The compound, which was discovered by Laboratoire L. Lafon (Boivin et al., 1993), is already on the market in France (under the tradename Modiodal).

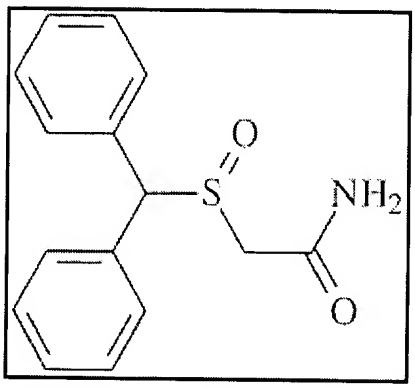


**Fig. 1.** *Structure of modafinil.*

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In clinical use, modafinil is likely to be administered in combination with other medications, and an understanding of the potential for drug-drug interactions is therefore very important. Such interactions could be pharmacokinetic or pharmacodynamic in nature, or both. The present study focused on potential pharmacokinetic interactions arising from inhibition or induction of cytochrome P450 drug-metabolizing enzymes by modafinil.

In a previous in vitro study in primary human hepatocytes (Moachon et al., 1996), modafinil was evaluated for its ability to induce the activities of cytochrome P450 enzymes, including ethoxyresorufin *O*-deethylase, pentoxyresorufin *O*-dealkylase, *S*-mephenytoin 4'-hydroxylase, dextromethorphan *O*-demethylase, nifedipine oxidase, and lauric acid hydroxylase. At concentrations (10 and/or 100 μM) approximating those achieved clinically, modafinil was found to induce ethoxyresorufin *O*-deethylase and nifedipine oxidase activities in human hepatocytes, although the extent of induction observed was less than that obtained in mice, rats, or dogs and less than those produced by known inducers of these cytochrome P450 enzymes. At the highest concentration tested (1000 μM), more pronounced changes were observed in the activities of ethoxyresorufin *O*-deethylase (increased), dextromethorphan *O*-demethylase (increased), and nifedipine oxidase (decreased) relative to those in the solvent-treated controls. The activity of *S*-mephenytoin 4'-hydroxylase was decreased at all concentrations of modafinil. The conclusion from this study was that ethoxyresorufin *O*-deethylase [cytochrome P450 (CYP)$^2$1A]

and nifedipine oxidase (CYP3A) activities were slightly induced by modafinil, which also increased the activity of dextromethorphan demethylase (CYP2D6) at the highest concentration tested (1000 μM).

Drawing definitive conclusions from these results was complicated by the substantial degree of intersubject variability that was observed and by the fact that modafinil was probably still present in the hepatocytes during the assay of enzymatic activities. In addition, the highest concentration tested (1000 μM) was substantially higher than the aqueous solubility of modafinil and was well above the concentration range obtained clinically (Wong et al., 1999).

A second in vitro induction study in human hepatocytes was therefore conducted to test whether the earlier results could be confirmed in a different laboratory, using a somewhat different and extended experimental design. In addition, the ability of modafinil to inhibit cytochrome P450 enzymes was studied in vitro in human liver microsomes (HLMs). The results of these latter two in vitro studies form the basis for the present communication.

## Materials and Methods


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**Chemicals, Enzymes, and Antibodies.** Modafinil was supplied by Cephalon, Inc. (West Chester, PA). Rifampin, α-naphthoflavone, nicotine, quinidine, 4-methylpyrazole, ketoconazole, baccatin, and 8-methoxypsoralen were purchased from Sigma Chemical Co. (St. Louis, MO). 7-Ethoxy-4-trifluoromethylcoumarin (EFC) was obtained from Molecular Probes (Junction City, OR). Paclitaxel and 6α-hydroxypaclitaxel were obtained from Hauser Chemical Co. (Boulder, CO) and Gentest Corp. (Woburn, MA), respectively. *S*-Mephenytoin, (±)-4'-hydroxymephenytoin, and hydroxymethyltolbutamide were purchased from Ultrafine Chemicals (Manchester, England). Furafylline was obtained from Research Biochemicals Inc. (Natick, MA). Hexobarbital was purchased from Sterling-Winthrop (Rensselaer, NY). Sulfaphenazole was obtained from Ciba-Geigy Ltd. (Basel, Switzerland). Troleandomycin was obtained from Pfizer, Inc. (Brooklyn, NY). Sources of other chemicals, including culture media components, were as specified by Pearce et al. (1996a) and Madan et al. (1999).

HLMs (individual livers), previously assayed for their activities of cytochrome P450 enzymes, were provided by XenoTech, L.L.C. (Kansas City, KS). cDNA-expressed enzymes were purchased from Gentest Corp. (Woburn, MA).

Purified polyclonal antibodies (rabbit-derived) for CYP1A1/CYP1A2, for CYP2B6, and for CYP3A4/CYP3A5 and monoclonal antibodies (mouse-derived) for CYP2A6/CYP2C8/CYP2C19, used in Western immunoblotting analysis, were commercial products provided by XenoTech, L.L.C.

**Human Hepatocytes.** Hepatocytes were isolated from human liver tissue obtained as surgical waste or from rejected donor livers via a modification of the two-step collagenase digestion method (Seglen et al., 1980; Quistorff et al., 1989; LeCluyse et al., 1996). Briefly, human liver tissue was perfused with pH 7.4 buffer containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, 5.5 mM

glucose, and 0.5 mM EGTA, followed by the same buffer lacking EGTA but containing 1.5 mM $CaCl_2$ and 0.2-0.5 mg/ml collagenase. Viability (trypan blue exclusion) was ≥70% for all preparations used.

**Enzyme Inhibition.** *Direct inhibition.*

Modafinil was incubated with HLMs (pool of seven subjects) at concentrations up to 250 μM. Significantly higher concentrations could not be tested due to the limited aqueous solubility of the compound. Modafinil was added in DMSO (final concentration, 0.1%), except for assay of CYP2E1, which is strongly inhibited by DMSO. For CYP2E1, modafinil was dissolved directly in the buffer mixture.

The substrates used for each enzyme and the concentrations tested (representing $K_m/2$, $K_m$, and $4K_m$) are presented in Table 1. A reference inhibitor for each enzyme, when available, was also included as a positive control.

**TABLE 1**

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*Summary of the cytochrome P450 enzyme assay conditions*

Incubations without DMSO solvent served as additional negative controls. The effects of the DMSO were minimal, except on 6β-hydroxylation of testosterone (CYP3A4/5), whose rates were decreased by up to 19% in the DMSO control. This extent of inhibition was not considered sufficient to compromise the results with modafinil.

*Metabolism-dependent (mechanism-based) inhibition.* To test for reversible inhibition, HLMs (two individual samples and a pool of seven) were preincubated with modafinil plus NADPH for 15 min before the addition of the substrate to start the assay. The concentrations of modafinil and of the substrates investigated are summarized in Table 1. Solvent controls, containing all components except modafinil, were also examined.

To test for irreversible inhibition, modafinil was incubated for 15 min with HLMs as was done for reversible inhibition but at 10- to 20-fold higher protein concentrations. Before assay, each microsomal mixture was diluted 10- to 20-fold to reduce any effects from reversible inhibition by modafinil or its metabolites.

**Enzyme Induction.** Freshly isolated human hepatocytes were cultured according to the method described by LeCluyse et al. (1994, 1996). Approximately $3 \times 10^6$ cells were added to 60-mm culture dishes coated with collagen and allowed to attach for 2 to 3 h. Unattached cells were aspirated, and serum-free modified Chee's medium containing 0.1 μM dexamethasone, 1% insulin-transferrin-selenium premix, and 0.25 mg/ml Matrigel was added. The cells were then maintained in culture for ~3 days, with daily changes of medium, before the initiation of experiments. Only preparations that contained morphologically normal hepatocytes, as evaluated by phase contrast light microscopy, without significant contamination from other cell types were treated with modafinil or reference inducers.

Hepatocytes were treated with modafinil at varying concentrations or with β-naphthoflavone (33 μM), phenobarbital (250 μM), or rifampicin (50 μM) to serve as positive controls. The final concentration of the solvent (DMSO) in the medium was 0.1%. Treatment was continued for 3 days, with daily renewal of the medium plus the test drug or reference compound.

Before harvest, hepatocytes were photographed to document the status of the cells after treatment. Approximately 24 h after the final treatment, the hepatocytes were rinsed and collected, and microsomes were prepared (Madan et al., 1999). Resuspension was in 0.25 M sucrose at a protein concentration of 1 to 10 mg/ml (BCA Protein Assay Kit; Pearce Chemical Co., Rockford, IL), with storage at −80°C, to preserve the activity of the cytochrome P450 enzymes (Pearce et al., 1996a).

**Assay Procedures.** Assays were performed as described in detail by Pearce et al. (1996a) or as described later. In each assay described later, the reaction mixture volume was 1 ml, containing 50 μg of microsomal protein, and the reactions were carried out at 37°C.

The equipment used for HPLC analysis in the paclitaxel and diclofenac assays included a Shimadzu LC-6A or LC-10A binary gradient HPLC system with an SIL-6A or SIL-10A autosampler and an SPD-6A or SPD-10A variable-wavelength UV detector. The column for both assays was a Supelcosil LC-18 reverse phase octyldecylsilane column (5 μm particle size; 4.6 mm i.d. × 15 cm) preceded by a Supelcosil LC-18 guard column (40 μm particle size; 4.6 mm i.d. × 2 cm) (Supelco, Bellefonte, PA). Column temperature was maintained at 30 ± 1°C with a CH-30 column heater controlled with a TC-50 temperature controller (Eppendorf, Inc., Madison, WI).

The *O*-dealkylation of EFC was measured using a modification of the fluorophotometric method of Buters et al. (1993). EFC (25 μM) was added in 5 μl of DMSO. Reactions proceeded for 5.0 min and were stopped by the addition of 2.0 ml of ice-cold acetone. Precipitated protein was removed by centrifugation. Concentrations of 7-hydroxy-4-trifluoromethylcoumarin in the supernatant were determined with a Shimadzu RF-540 spectrofluorometer ($\lambda_{ex}$ = 410 nm; $\lambda_{em}$ = 510 nm). Zero time incubations served as blanks, and blanks spiked with 20 to 1000 pmol of 7-hydroxy-4-trifluoromethylcoumarin served as standards.

The oxidation of paclitaxel was monitored by reverse phase HPLC, based on the method described by Richheimer et al. (1992) and Cresteil et al. (1994), with slight modifications. Paclitaxel (10 μM) was added in 10 μl of acidic methanol. Reactions were started by the addition of an NADPH-generating solution and were stopped after 60 min by the addition of 5.9 ml of dichloromethane. Zero time incubations served as blanks, and blanks spiked with 120 to 1200 pmol of 6α-hydroxypaclitaxel (added in 40 μl of methanol), as standards. Each sample was spiked with 3 nmol of the internal standard, baccatin (in 100 μl of dichloromethane), and vigorously mixed on a batch vortexer. After the two phases were separated by low-speed centrifugation, the aqueous (upper) phase was aspirated and discarded. An aliquot (4 ml) of the organic phase was transferred to a culture tube and evaporated in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY). The residue was redissolved in 200 μl of mobile phase, and a 50-μl aliquot was analyzed by HPLC. The isocratic mobile phase was water/acetonitrile 60:40 (v/v), at a total flow rate of 1.5 ml/min. Eluates were monitored at 235 nm. Total analysis time was

20 min/run, and the retention times for baccatin, 6α-hydroxypaclitaxel, and paclitaxel were ~3.4, ~8.6, and ~15.0 min, respectively. Paclitaxel and 6α-hydroxypaclitaxel were quantified by peak area compared with authentic standards, with correction for variation in extraction efficiency based on recovery of the internal standard.

The 4'-hydroxylation of diclofenac (100 μM) was measured by reverse phase HPLC, based on the method described by Leemann et al. (1993). Reactions were started by the addition of an NADPH-generating system and were stopped after 30 min by addition of 1.0 ml of methanol. Precipitated protein was removed by low-speed centrifugation, and a 400-μl aliquot of the supernatant fraction was analyzed by HPLC. Zero time incubations served as blanks, and blanks spiked with 50 to 1000 pmol of 4'-hydroxydiclofenac (added in 20 μl of methanol) served as standards. The isocratic mobile phase was 20 mM potassium phosphate buffer (pH 7.0)/acetonitrile 75:25 (v/v), at a total flow rate of 1.0 ml/min. Total analysis time was 15 min/run, and the retention times of 4'-hydroxydiclofenac and diclofenac were ~3.7 and ~11.0 min, respectively. Eluates were monitored at 282 nm. 4'-Hydroxydiclofenac was quantified using peak area compared with authentic standards.

**Western Immunoblotting Procedures.** CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C19, and CYP3A4 in the hepatocyte-derived microsomes were analyzed via Western immunoblotting using essentially the procedures described by Madan et al. (1999).

**Data Analysis.** All incubations for enzyme activity assays were in duplicate; the data reported are averages of those duplicate determinations.

The results of the inhibition study were analyzed by Dixon and Eadie-Hofstee plots to determine the type of inhibition and the value of the inhibitory constant ($K_i$). If an enzyme was not inhibited at the highest concentration of modafinil tested, an estimated minimum value of $K_i$ was calculated using the following equation for competitive inhibition (Todhunter, 1979):

$$K_i = \frac{V[I][K_m]}{V_{max}[S] - V(K_m - [S])}$$

where [I] is the concentration of modafinil and [S] is the concentration of the marker substrate. Assuming under experimental conditions that would be most sensitive to inhibition (i.e., [I] = 250 μM; [S] = $K_m$/2) that a 10% inhibition would be detectable within experimental variability, the minimum $K_i$ value was estimated to be 1500 μM.

To determine significant differences between group mean values in the induction study, data were first determined to be parametrically distributed; then, a one-way ANOVA test for repeated measures was carried out. When significance ($P < .05$) was observed, a Dunnett's post hoc test was used to identify the group mean values that were significantly different from the controls ($P < .05$).

## Results

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**Inhibition of Human Cytochromes P450.** The rates of metabolism of marker substrates for nine cytochrome P450 enzymes (Table 1) were determined in HLMs (pool of seven) in the presence and absence of modafinil (5-250 μM). The presence of modafinil had minimal effect on any reaction except the 4'-hydroxylation of *S*-mephenytoin (CYP2C19), which was reversibly inhibited with a $K_i$ value of ~39 μM (Fig. 2). This concentration approximates the steady-state $C_{max}$ value of modafinil in human plasma at a dosage of 400 mg/day (Wong et al., 1999). The sample-to-sample variation in the inhibition of CYP2C19 was determined with 10 samples of individual HLMs at substrate concentrations equal to $K_m$ and modafinil concentrations equal to ~2$K_i$. As expected, modafinil produced substantial inhibition (~50%) of CYP2C19 in all microsomal samples (Fig. 3), consistent with its ability to function as a competitive inhibitor.

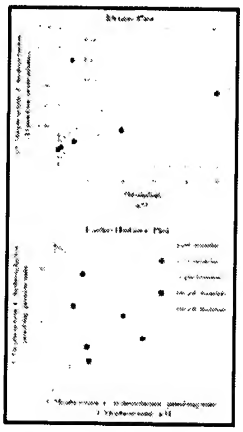

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**Fig. 2.** *Evaluation of modafinil as a reversible inhibitor of S-mephenytoin 4'-hydroxylase (CYP2C19) activity in pooled HLMs.*

Dixon and Eadie-Hofstee plots of the rates of *S*-mephenytoin 4'-hydroxylation by pooled HLMs. The microsomes were incubated with *S*-mephenytoin for 30 min at concentrations of the substrate that were approximately equal to $K_m/2$, $K_m$, and $4K_m$ and in the absence (solvent control; 0.1% DMSO) or presence of modafinil at concentrations of 5, 25, 100, or 250 μM.

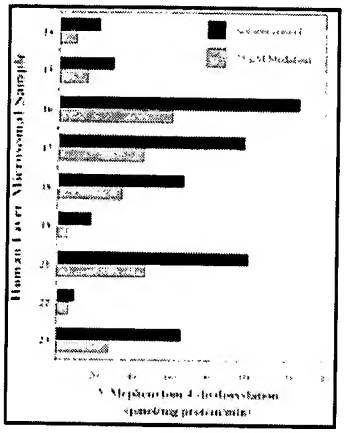

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**Fig. 3.** *Sample-to-sample variation in the inhibition of S-mephenytoin 4'-hydroxylase (CYP2C19) activity in HLMs by modafinil.*

The rates of *S*-mephenytoin 4'-hydroxylation were measured in the presence and absence (solvent control; 0.1% DMSO) of modafinil in individual 30-min incubations of microsomes prepared from nine different human livers. The substrate concentration was equal to $K_m$, and the concentration of modafinil, when present, was 75 μM.

In addition, CYP3A4/5 and CYP2B6 were weakly inhibited. 6β-Hydroxylation of testosterone (CYP3A4/5) was uncompetitively inhibited with a $K_i$ value of ~632 μM (Fig. 4), and *O*-dealkylation of EFC (CYP2B6) was noncompetitively inhibited with a $K_i$ value of ~1200 μM (data not shown). However, these $K_i$ values are both much higher than the concentrations of modafinil attained clinically (Wong et al., 1999). The other cytochrome P450 activities appeared to be unaffected ($K_i$ > 1500 μM) by the presence of modafinil at the concentrations tested.

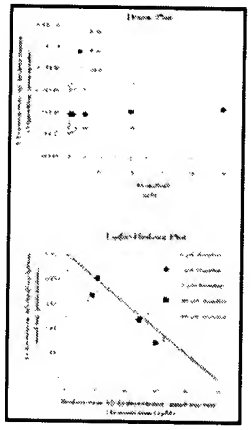

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**Fig. 4.** *Evaluation of modafinil as a reversible inhibitor of testosterone 6β-hydroxylase (CYP3A4/5) activity in pooled HLMs.*

Dixon and Eadie-Hofstee plots of testosterone 6β-hydroxylation by pooled HLMs. The microsomes were incubated with testosterone for 8 min at concentrations of the substrate that were approximately equal to $K_m/2$, $K_m$, and $4K_m$ and in the absence (solvent control; 0.1% DMSO) or presence of modafinil at concentrations of 5, 25, 100, or 250 μM.

The ability of metabolites of modafinil to affect the activities of hepatic drug-metabolizing enzymes was examined by preincubating the microsomes and cofactors with modafinil before the addition of each marker substrate and then comparing the rates of metabolism obtained under those conditions with the rates obtained when the substrate was introduced at the same time as modafinil. No reversible or irreversible metabolism-dependent inhibition was observed. A representative data set displaying the

effect of modafinil on testosterone 6β-hydroxylase activity (CYP3A4/5) is shown in Fig. 5.

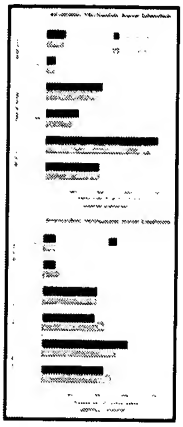

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**Fig. 5.** *Evaluation of modafinil as a metabolism-dependent (mechanism-based) inhibitor of testosterone 6β-hydroxylase (CYP3A4/5) activity in HLMs.*

HLMs (two individual samples and a pool of seven) were incubated with modafinil (250 μM) or with the solvent only (DMSO; final concentration, 0.1%) for 15 min under the assay conditions before starting the assay reaction by introduction of the marker substrate, testosterone (50 μM). After 8 min, the reaction was quenched, and the rate of formation of 6β-hydroxytestosterone was calculated. The rates without preincubation were then compared with those after incubation for both the modafinil-treated microsomes and the solvent controls. To test for reversible inhibition, the substrate was added to undiluted microsomal mixtures. To test for irreversible inhibition, the preincubation was conducted at microsomal protein concentrations that were 10- to 20-fold higher than those normally used in the CYP3A4/5 assay. Immediately before the addition of the substrate, these microsomal mixtures were diluted 10- to 20-fold to reduce any effect from reversible inhibition.

**Induction of Human Cytochromes P450.** The ability of modafinil to induce cytochrome P450 activities was examined in vitro in primary cultures of human hepatocytes. After ~3 days in culture, the hepatocytes were exposed to modafinil at concentrations of 10 to 300 μM for 3 additional days. The hepatocytes were then harvested, and microsomes were prepared and assayed for the activities of eight cytochrome P450 enzymes. The mean results are presented in Fig. 6. [Note: Due to low hepatocyte yield from one human liver (HL-5), the 100 μM treatment group was omitted for that liver to ensure that the other treatment conditions could be effectively evaluated.]

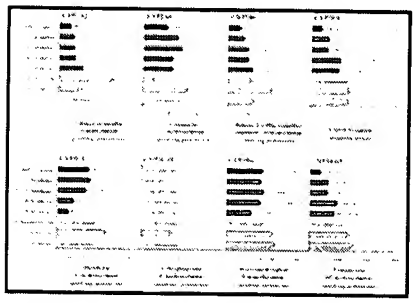

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**Fig. 6.** *Effect of treating primary human hepatocyte cultures with modafinil and prototypical cytochrome P450 enzyme inducers on the activities of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, and CYP3A4/5 and on the protein level of CYP2C8.*

Human hepatocytes from five donors (HL-1 to HL-5) were placed in culture for 3 days before the initiation of treatment with modafinil at 0 (solvent control), 10, 30, 100, or 300 μM for 3 days, with renewal of the medium and test article every 24 h. Additional culture dishes were similarly exposed to β-naphthoflavone (33 μM), phenobarbital (250 μM), or rifampicin (50 μM) and served as positive controls. The final concentration of the solvent (DMSO) in the medium was 0.1%. Microsomes were prepared for each human liver from each of the treatment groups. The enzymatic activity of each of the CYP enzymes was then determined for each liver and treatment group using marker substrates. In the case of CYP2C8, the rate of 6α-hydroxylation of paclitaxel, the marker substrate, was too low to be measured; hence, the levels of CYP2C8 protein (Western immunoblots) were used to assess induction. For each assay, there were five determinations per treatment group, unless otherwise noted above the bar. Values represent mean ± S.D. $*P < .05$ in the comparison of modafinil-treated samples with the corresponding DMSO controls. $\dagger P < .05$ in the comparison of all treated samples with the corresponding DMSO controls.

No significant changes were observed in the activities of CYP2A6, CYP2C8, CYP2C19, or CYP2D6 (Fig. 6). However, the activities of CYP2C19 in the microsomes from modafinil-treated and solvent control hepatocytes were below the limits of detection of the assay; only the positive controls produced detectable levels of enzyme activity. Hence, an induction of CYP2C19 to levels less than those in the positive controls might not have been detected.

The enzymatic activity of CYP2C8 was also below the limit of detection in all microsomal samples, except those from one phenobarbital-treated control. However, CYP2C8 protein was detected in all of the microsomal preparations by Western immunoblotting. The levels of the protein were low relative to those in microsomes prepared directly from liver and were not consistent within or between the hepatocyte preparations and treatment groups. An apparent, slight increase in mean CYP2C8 protein concentration was observed at the two highest concentrations of modafinil, but the differences between the solvent control and modafinil-treated preparations were not statistically significant ($P > .05$). Although a weak induction of CYP2C8 by high concentrations of modafinil cannot be ruled out on the basis of these data, the likelihood of a substantive effect appears to be low.

The enzymatic activities associated with CYP3A4/5 and CYP1A2 were increased slightly, in a generally concentration-related manner, over those in the solvent-treated controls (Fig. 6). The activity of EFC

*O*-deethylase, a marker for CYP2B6, was also increased, but this compound is also a substrate for human CYP1A2. Therefore, at least part of the increase in EFC *O*-deethylase activity reflected the induction of CYP1A2.

The activity of CYP2C9 (which was not examined in the previous study reported by Moachon et al., 1996) was decreased up to 60% in modafinil-treated hepatocytes relative to the activity in solvent-only treated cells (Fig. 6). The decrease was concentration-dependent but did not represent an appreciable change in activity except at concentrations of modafinil of ≥100 μM.

To determine whether the changes in enzyme activity were due to changes in enzyme concentration, Western immunoblotting was carried out with antibodies to CYP1A2, CYP2A6/2C8/2C19, CYP2B6, and CYP3A4/3A5. The results for CYP1A2 and CYP3A4 were consistent with those obtained by determination of enzymatic activity. A picture of two representative Western immunoblots of CYP3A4 protein, representing three of the hepatocyte preparations, is shown in Fig. 7. In addition, the immunoblots showed modest induction of CYP2B6, whose level of induction could not be determined solely from the enzymatic assay.

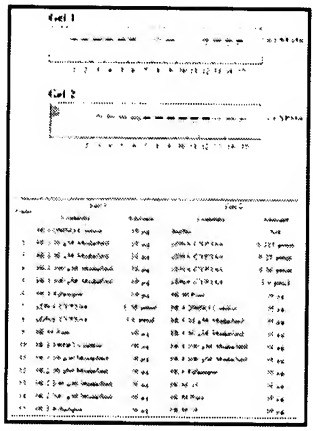

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**Fig. 7.** *Effect of treating primary cultures of human hepatocytes with modafinil on the level of CYP3A4 expression (Western immunoblotting).*

Human hepatocytes from five donors (HL-1 to HL-5) were placed in culture for 3 days before initiation of treatment with modafinil at 0 (solvent control), 10, 30, 100, or 300 μM for 3 days, with renewal of the medium and test article every 24 h. Additional culture dishes were similarly exposed to β-naphthoflavone (33 μM), phenobarbital (250 μM), or rifampicin (50 μM) and served as positive controls. The final concentration of the solvent (DMSO) in the medium was 0.1%. Microsomes were prepared for each human liver from each of the treatment groups, and a portion of each was subjected to Western immunoblotting to measure the levels of immunoreactive CYP3A4 using a polyclonal antibody against rat CYP3A1. For comparison, HLMs prepared directly from the livers of two individual subjects (HLM15 and HLM16), plus a pool of microsomes, were also evaluated, in addition to known concentrations of cDNA-derived CYP3A4.

## Discussion


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In the present study, the ability of modafinil to inhibit or to induce cytochrome P450 enzyme activities was studied in human liver preparations. Such information is important for the design of effective treatment programs that will include administration of modafinil, because such enzymatic interactions can either enhance or diminish the effectiveness and/or safety of concomitant medications.

Cytochrome P450 inhibition by modafinil appears to be limited to CYP2C19, whose substrates and inhibitors have been extensively reviewed (e.g., Flockhart, 1995; Parkinson, 1996; Rendic and Di Carlo, 1997). Modafinil does not itself appear to be a substrate for CYP2C19, and there are a relatively small number of marketed pharmaceutical products that are predominantly or even largely metabolized by the enzyme. Examples are *S*-mephenytoin (Goldstein et al., 1994), omeprazole (Ko et al., 1997), lansoprazole (Pearce et al., 1996b), proguanil (Wright et al., 1995), diazepam (Jung et al., 1997), and propranolol (Ward et al., 1989). (Omeprazole and lansoprazole are also potent inhibitors of CYP2C19.) Caution should be exercised when initiating cotherapy with modafinil in patients receiving these medications, but with the possible exception of diazepam or other sedative benzodiazepines, they generally are not drugs with which modafinil is likely to be a frequent comedication.

However, as demonstrated by a recent report (Grözinger et al., 1998) of an apparent metabolic drug-drug interaction of modafinil with clomipramine, inhibition of CYP2C19 could, in special cases, also be important for compounds that are not normally considered to be significant substrates for the enzyme. In the case reported, the plasma concentrations of clomipramine were found to have increased, along with those of its pharmacologically active *des*-methyl metabolite, after the addition of modafinil as a comedication. Because clomipramine and *des*-methylclomipramine normally are largely eliminated through metabolism by CYP2D6 (Nielsen et al., 1992, 1996), a significant effect by an inhibitor of CYP2C19 was unexpected. However, the patient was subsequently determined to be CYP2D6-deficient (Grözinger et al., 1998), belonging to a subset of the human population who have no functional CYP2D6 enzyme (i.e., 7-10% of whites and equal or smaller portions of other ethnic groups; Eichelbaum, 1984; Setiabudy et al., 1994). In these "poor metabolizers" of CYP2D6 substrates, such as dextromethorphan, debrisoquine, and sparteine, the fractional contributions of alternative metabolic pathways for clomipramine and *des*-methylclomipramine through CYP2C19 and other cytochrome P450 enzymes could assume substantially more important roles than would be the case in individuals with normal CYP2D6 activity.

The inhibition of CYP2C19 by modafinil would likely have minimal therapeutic consequences for patients at steady state for modafinil and for whom a tricyclic antidepressant would be prescribed as cotherapy, because the dosage of the antidepressant would generally be titrated to identify a safe and effective dose. However, these results would suggest that in patients at steady state for clomipramine or similar tricyclic antidepressants, the addition of modafinil as cotherapy may require a dosage reduction for the antidepressant, particularly in CYP2D6-deficient individuals.

Modafinil also caused an induction of cytochrome P450 activities in vitro in human hepatocytes. Three enzymes appeared to be induced by modafinil: CYP1A2, CYP3A4, and CYP2B6. [The results for CYP1A2 and CYP3A4 were generally consistent with results obtained previously (Moachon et al., 1996); CYP2B6 was not previously examined.] The extent of induction of each enzyme, although statistically significant at one or more concentrations of modafinil, was modest, especially in comparison with those produced by reference inducers, when available, and in comparison with interindividual variability.

No significant effect of modafinil treatment (10-300 μM) was observed in the activities of CYP2A6,

CYP2C8, CYP2C19, or CYP2D6. The apparent suppression of CYP2C19 reported previously (Moachon et al., 1996) was likely due to inhibition of the enzyme by residual modafinil that remained in the hepatocyte preparations during the enzymatic assays. The lack of induction of CYP2D6 in the present study at concentrations of up to 300 μM is consistent with the previous results for all except the highest concentration tested in that study (i.e., 1000 μM). The reason for the increased enzymatic activity at that concentration is not known, but the concentration is far beyond those that have any clinical relevance.

Of the three cytochrome P450 enzymes apparently inducible by modafinil, CYP1A2 and CYP2B6 do not appear to be of major concern. CYP1A2 provides the primary metabolic pathway for relatively few pharmacologically important substrates, and these do not have narrow therapeutic indices (Tassaneeyakul et al., 1993; Brøsen, 1995; Bertz and Granneman, 1997). In addition, the extent of induction observed in the present study, or in the previous one (Moachon et al., 1996), was small relative to the ~40-fold interindividual variability observed for this enzyme. In the case of CYP2B6, the activity of the enzyme is extremely low in human livers (Shimada et al., 1994), and it appears to contribute minimally to the metabolism of pharmaceutical products.

In contrast, CYP3A4 represents the largest single portion of the cytochrome P450 protein and activity in the human liver and plays a substantial role in the metabolism of a vast array of pharmaceutical products (Guengerich, 1995; Parkinson, 1996; Bertz and Granneman, 1997; Rendic and Di Carlo, 1997). Of particular concern are compounds that are predominantly or exclusively metabolized by CYP3A4 and also have a narrow therapeutic margin (e.g., cyclosporine A and steroidal contraceptives containing ethinyl estradiol).

As with the inhibition of CYP2C19, the apparently low degree of induction of CYP3A4 by modafinil would be most likely to produce clinical effects if modafinil were added as cotherapy to a patient already at steady state for a narrow-margin CYP3A4 substrate. A single case of apparent interaction has been reported in a patient in whom the effectiveness of treatment with cyclosporine A decreased after the addition of modafinil as a cotherapy (Le Cacheux et al., 1997). However, insufficient information is available to establish the cause of the effect.

Finally, the apparent suppression of CYP2C9 activity in human hepatocytes by treatment with modafinil is potentially important due primarily to one compound, warfarin (Coumadin), which has a narrow therapeutic index and whose more active enantiomer (*S*-warfarin) is primarily a substrate for CYP2C9 (Rettie et al., 1992). The origin of the suppressive effect obtained in vitro and its relevance to the clinical situation are not known, but the finding suggests caution in the initiation of treatment with modafinil in patients who are at steady state on warfarin.

In summary, modafinil has been demonstrated in vitro to be a moderately potent, reversible inhibitor of CYP2C19 in HLMs and a modest inducer of CYP1A2, CYP3A4, and CYP2B6 in vitro in human hepatocytes. In addition, CYP2C9 appeared to be suppressed in vitro in human hepatocytes after treatment with modafinil. Overall, these results suggest that there is potential for metabolic drug-drug interactions between modafinil and certain possible concomitant medications.

Due to the relatively low degree of alteration of the enzyme activities in vitro and to the concentrations

of modafinil required to obtain appreciable effects, a high incidence of clinically significant interactions would not be expected. However, these in vitro results are being used in evaluation of clinical reports of apparent drug-drug interactions and in the design of subsequent studies targeted at further elucidation of the clinical relevance, if any, of these in vitro findings.

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## Footnotes

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A preliminary report of this study was presented as a poster at the 12th International Symposium on Microsomes and Drug Oxidations, July 20-24, 1998, in Montpellier, France, Abstract 314.

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## Abbreviations

Abbreviations used are: CYP, cytochrome P450; EFC, 7-ethoxy-4-trifluorocoumarin; 7-ethoxyresorufin, 7-ethoxyphenoxazone; HLM, human liver microsomes; 6β-hydroxytestosterone, 4-androsten-6β,17β-diol-3-one; resorufin, 7-hydroxyphenoxazone; testosterone, 4-androsten-17β-ol-3-one.

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DRUG METABOLISM AND DISPOSITION


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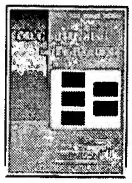

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A. Madan, R. A. Graham, K. M. Carroll, D. R. Mudra, L. A. Burton, L. A. Krueger, A. D. Downey, M. Czerwinski, J. Forster, M. D. Ribadeneira, L.-S. Gan, E. L. LeCluyse, K. Zech, P. Robertson Jr., P. Koch, L. Antonian, G. Wagner, L. Yu, and A. Parkinson

**Effects of Prototypical Microsomal Enzyme Inducers on Cytochrome P450 Expression in Cultured Human Hepatocytes**

Drug Metab. Dispos., April 1, 2003; 31(4): 421 - 431.

[Abstract] [Full Text] [PDF]

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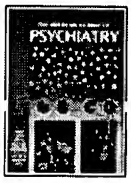

THE AMERICAN JOURNAL OF PSYCHIATRY ▸HOME

J. R. DEQUARDO

**Modafinil-Associated Clozapine Toxicity**

Am. J. Psychiatry, July 1, 2002; 159(7): 1243 - 1244.

[Full Text]

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medicament for therapeutic or prophylactic treatment of a disease or medical condition in which **cathepsin K** is implicated.

9. A process for the preparation of a compound of formula I or a salt or ester thereof which comprises coupling the corresponding Het substituted benzoic acid derivative of formula III ##STR8## wherein Het is as defined in claim 1, with 1-amino-cyclohexanecarboxylic acid cyanomethyl-amide.

PI US 2001016207 A1 20010823

L32 ANSWER 7 OF 9 USPATFULL

AB Dipeptide nitrile **Cathepsin K inhibitors** of formula I, and pharmaceutically acceptable salts or esters thereof ##STR1##

In which

R.sub.1 and R.sub.2 are independently H or C.sub.1-C.sub.7lower alkyl, or R.sub.1 and R.sub.2 together with the carbon atom to which they are attached form a C.sub.3-C.sub.8cycloalkyl ring, and

Het is an optionally substituted nitrogen-containing heterocyclic substituent, are provided, useful e.g. for therapeutic or prophylactic treatment of a disease or medical condition in which **cathepsin K** is implicated.

CLM What is claimed is:

1. A compound of formula I, or a pharmaceutically acceptable salt or ester thereof ##STR6## In which R.sub.1 and R.sub.2 are independently H or C.sub.1-C.sub.7 lower alkyl, or R.sub.1 and R.sub.2 together with the carbon atom to which they are attached form a C.sub.3-C.sub.8cycloalkyl ring, and Het is an optionally substituted nitrogen-containing heterocyclic substituent, provided that Het is not 4-pyrrol-1-yl.

2. A compound according to claim 1 of formula II, or a pharmaceutically acceptable salt or ester thereof ##STR7## wherein X is CH or N, and R is C.sub.1-C.sub.7lower alkyl, C.sub.1-C.sub.7lower alkoxy-C.sub.1-C.sub.7lower alkyl, C.sub.5-C.sub.10aryl-C.sub.1-C.sub.7lower alkyl, or C.sub.3-C.sub.8cycloalkyl.

3. A compound according to claim 1, or a pharmaceutically acceptable salt or ester thereof, selected from N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-methyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-ethyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[4-(1-propyl)-piperazin-1-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-isopropyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(4-benzyl-piperazin-1-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[4-(2-methoxy-ethyl)-piperazin-1-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-propyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[1-(2-methoxy-ethyl)-piperidin-4-yl]-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-isopropyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-cyclopentyl-piperidin-4-yl)-benzamide; N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(1-methyl-piperidin-4-yl)-benzamide, or N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-(piperidin-4-yl)-benzamide.

4. N-[1-(Cyanomethyl-carbamoyl)-cyclohexyl]-4-[4-(1-propyl)-piperazin-1-yl]-benzamide, or a pharmaceutically acceptable salt or ester thereof.

5. A compound according to claim 1 for use as a pharmaceutical.

6. A pharmaceutical composition comprising a compound according to claim 1 as an active ingredient.

7. A method of treating a patient suffering from or susceptible to a disease or medical condition in which **cathepsin K** is implicated, comprising administering an effective amount of a compound according to claim 1 to the patient.

8. The use of a compound according to claim 1 for the preparation of a

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22. A pharmaceutical **composition** comprising a compound according to claim 1 and a pharmaceutically acceptable carrier.

23. A pharmaceutical **composition** made by combining a compound according to claim 1 and a pharmaceutically acceptable carrier.

24. A process for making a pharmaceutical **composition** comprising combining a compound according to claim 1 and a pharmaceutically acceptable carrier.

25. The **composition** of claim 22 which further comprises an active ingredient selected from the group consisting of a) an organic bisphosphonate or a pharmaceutically acceptable salt or ester thereof, b) an estrogen receptor modulator, c) a cytotoxic/antiproliferative agent, d) a matrix metalloproteinase inhibitor, e) an inhibitor of epidermal-derived, fibroblast-derived, or platelet-derived growth factors, f) an inhibitor of VEGF, g) an inhibitor of Flk-1/KDR, Flt-1, Tck/Tie-2, or Tie-1, h) a **cathepsin K** inhibitor, and i) a prenylation inhibitor, such as a farnesyl transferase inhibitor or a geranylgeranyl transferase inhibitor or a dual farnesyl/geranylgeranyl transferase inhibitor; and mixtures thereof.

26. The **composition** of claim 25 wherein said active ingredient is selected from the group consisting of a) an organic bisphosphonate or a pharmaceutically acceptable salt or ester thereof, b) an estrogen receptor modulator, and c) a **cathepsin K** inhibitor; and mixtures thereof.

27. The **composition** of claim 26 wherein said organic bisphosphonate or pharmaceutically acceptable salt or ester thereof is alendronate monosodium trihydrate.

28. The **composition** of claim 25 wherein said active ingredient is selected from the group consisting of a) a cytotoxic/antiproliferative agent, b) a matrix metalloproteinase inhibitor, c) an inhibitor of epidermal-derived, fibroblast-derived, or platelet-derived growth factors, d) an inhibitor of VEGF, and e) an inhibitor of Flk-1/KDR, Flt-1, Tck/Tie-2, or Tie-1; and mixtures thereof.

29. A method of eliciting an integrin receptor antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a compound according to claim 1.

30. The method of claim 29 wherein the integrin receptor antagonizing effect is an .alpha.v.beta.3 antagonizing effect.

31. The method of claim 30 wherein the .alpha.v.beta.3 antagonizing effect is selected from the group consisting of inhibition of bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, viral disease, and tumor growth.

32. The method of claim 31 wherein the .alpha.v.beta.3 antagonizing effect is the inhibition of bone resorption.

33. The method of claim 29 wherein the integrin receptor antagonizing effect is an .alpha.v.beta.5 antagonizing effect.

34. The method of claim 33 wherein the .alpha.v.beta.5 antagonizing effect is selected from the group consisting of inhibition of restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, and tumor growth.

35. The method of claim 29 wherein the integrin receptor antagonizing effect is a dual .alpha.v.beta.3/.alpha.v.beta.5 antagonizing effect.

36. The method of claim 35 wherein the dual .alpha.v.beta.3/.alpha.v.beta.5 antagonizing effect is selected from the group consisting of inhibition of bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, viral disease, and tumor growth.

37. The method of claim 29 wherein the integrin antagonizing effect is an .alpha.v.beta.6 antagonizing effect.

38. The method of claim 37 wherein the .alpha.v.beta.6 antagonizing effect is selected from the group consisting of angiogenesis, inflammatory response, and wound healing.

39. A method of eliciting an integrin receptor antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 22.

40. A method of treating or preventing a condition mediated by antagonism of an integrin receptor in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 22.

41. A method of inhibiting bone resorption in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 22.

42. A method of inhibiting bone resorption in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 26.

43. A method of treating tumor growth in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 28.

44. A method of treating tumor growth in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a compound according to claim 1 in combination with radiation therapy.

45. The compound of claim 21 selected from the group consisting of 3(S)-(2,3-Dihydro-benzofuran-6-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid, 3(S)-(Quinolin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid, 3(S)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid, 3(S)-(6-Ethoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid, 3(S)-(4-Ethoxy-3-fluorophenyl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]napthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid; and the pharmaceutically acceptable salts thereof.

46. The compound of claim 45 which is 3(S)-(2,3-Dihydro-benzofuran-6-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid or a pharmaceutically acceptable salt thereof.

47. The compound of claim 45 which is 3(S)-(6-Methoxypyridin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid or a pharmaceutically acceptable salt thereof.

48. The compound of claim 45 which is 3(S)-(Quinolin-3-yl)-3-{2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-propyl]-imidazolidin-1-yl}-propionic acid or a pharmaceutically acceptable salt thereof.

AB The present invention relates to compounds and derivatives thereof, their synthesis, and their use as integrin receptor antagonists. More particularly, the compounds of the present invention are antagonists of the integrin receptors .alpha.v.beta.3, .alpha.v.beta.5 and/or .alpha.v.beta.6 and are useful for inhibiting bone resorption, treating and preventing osteoporosis, and inhibiting vascular restenosis, diabetic retinopathy, macular degeneration, angiogenesis, atherosclerosis, inflammation, wound healing, viral disease, and tumor growth and metastasis.

ACCESSION NUMBER: 2000:9915 USPATFULL
TITLE: Integrin receptor antagonists
INVENTOR(S): Askew, Ben C., Lansdale, PA, United States
Coleman, Paul J., Wallingford, PA, United States
Duggan, Mark E., Schwenksville, PA, United States
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Hutchinson, John H., Philadelphia, PA, United States
Meissner, Robert S., Schwenksville, PA, United States
Patane, Michael A., Harleysville, PA, United States
Wang, Jiabing, Lansdale, PA, United States
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 6017926 | | 20000125 | |
| APPLICATION INFO.: | US 1998-212079 | | 19981215 | (9) |

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| PRIORITY INFORMATION: | US 1997-69910P | 19971217 | (60) |
| | US 1998-83251P | 19980427 | (60) |
| | US 1998-92588P | 19980713 | (60) |
| | US 1998-79197P | 19980324 | (60) |
| | US 1998-79944P | 19980330 | (60) |
| | US 1998-80397P | 19980402 | (60) |
| | US 1998-92624P | 19980713 | (60) |
| | US 1998-99948P | 19980911 | (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Dentz, Bernard
LEGAL REPRESENTATIVE: Durette, Philippe L., Winokur, Melvin, Sabatelli, Anthony D.
NUMBER OF CLAIMS: 48
EXEMPLARY CLAIM: 1
LINE COUNT: 5668
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 34 OF 34 USPATFULL
CLM What is claimed is:
1. A compound of formula: ##STR62## known as 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[4-(N,N-dimethylaminomethyl)benzyloxy]carbonyl-L-leucinyl]carbonhydrazide; or a pharmaceutically acceptable salt, hydrate or solvate thereof.

2. A pharmaceutical **composition** comprising a compound according to claim 1 and a pharmaceutically acceptable carrier, diluent or excipient.

3. A method of inhibiting a cysteine protease comprising administering to a patient in need thereof an effective amount of a compound according to claim 1.

4. A method according to claim 3 wherein said cysteine protease is **cathepsin K**.

5. A method of treating a disease characterized by bone loss comprising inhibiting said bone loss by administering to a patient in need thereof an effective amount of a compound according to claim 1.

6. A method according to claim 5 wherein said disease is osteoporosis.

7. A method according to claim 5 wherein said disease is periodontitis.

8. A method according to claim 5 wherein said disease is gingivitis.

9. A method of treating a disease characterized by excessive cartilage or matrix degradation comprising inhibiting said excessive cartilage or matrix degradation by administering to a patient in need thereof an effective amount of a compound according to claim 1.

10. A method according to claim 9 wherein said disease is osteoarthritis.

11. A method according to claim 9 wherein said disease is rheumatoid arthritis.

AB Disclosed herein is a compound of the formula ##STR1## known as 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[4-(N,N-dimethylaminomethyl)benzyloxy]carbonyl-L-leucinyl]carbohydrazide; and pharmaceutically acceptable salts, hydrates and solvates thereof.

ACCESSION NUMBER: 1999:160086 USPATFULL
TITLE: Protease inhibitors
INVENTOR(S): Halbert, Stacie Marie, Harleysville, PA, United States
Thompson, Scott Kevin, Phoenixville, PA, United States
Veber, Daniel Frank, Ambler, PA, United States
PATENT ASSIGNEE(S): SmithKline Beecham Corporation, Philadelphia, PA, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5998470 | | 19991207 |
| APPLICATION INFO.: | US 1999-290958 | | 19990413 (9) |
| RELATED APPLN. INFO.: | Division of Ser. No. US 793915 | | |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 1995-8108P | 19951030 (60) |
| | US 1995-7473P | 19951122 (60) |
| | US 1995-8992P | 19951221 (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Stockton, Laura L.
LEGAL REPRESENTATIVE: Stercho, Yuriy P., Venetianer, Stephen A., Kinzig, Charles M.
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
LINE COUNT: 6106
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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L16 ANSWER 31 OF 34 USPATFULL

CLM What is claimed is:

1. A compound of the formula ##STR18## wherein W is selected from the group consisting of a 5- or 6-membered monocyclic aromatic or nonaromatic ring system having 1, 2, 3 or 4 heteroatoms selected from the group consisting of N, O, and S wherein the ring nitrogen atoms are unsubstituted or substituted with one R.sup.1 substituent and the ring carbon atoms are unsubstituted or substituted with one or two R.sup.1 substituents, and a 9- to 1 4-membered polycyclic ring system, wherein one or more of the rings is aromatic, and wherein the polycyclic ring system has 1, 2, 3 or 4 heteroatoms selected from the group consisting of N, O, and S wherein the ring nitrogen atoms are unsubstituted or substituted with one R.sup.1 substituent and the ring carbon atoms are unsubstituted or substituted with one or two R.sup.1 substituents; X is selected from the group consisting of --(CH.sub.2).sub.v --, and --(CH.sub.2).sub.v NR.sup.4 (CH.sub.2).sub.v --; wherein any methylene (CH.sub.2) carbon atom, other than in R.sup.4, is either unsubstituted or substituted with one or two R.sup.3 substitutents; Y is ##STR19## wherein the biphenyl ring system is either unsubstituted or substituted with one or more R.sup.1 substituents; Z is selected from the group consisting of ##STR20## --CH.sub.2 CH.sub.2 -- and --CH.dbd.CH--, wherein either carbon atom can be substituted by one or two R.sup.3 substituents; each R.sup.1 is independently selected from the group consisting of hydrogen, halogen, C.sub.1-10 alkyl, C.sub.3-8 cycloalkyl, C.sub.3-8 cycloheteroalkyl, C.sub.3-8 cycloalkyl C.sub.1-6 alkyl, C.sub.3-8 cycloheteroalkyl C.sub.1-6 alkyl, aryl, aryl C.sub.1-8 alkyl, amino, amino C.sub.1-8 alkyl, C.sub.1-3 acylamino, C.sub.1-3 acylamino C.sub.1-8 alkyl, (C.sub.1-6 alkyl).sub.p amino, (C.sub.1-6 alkyl).sub.p amino C.sub.1-8 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxy C.sub.1-6 alkyl, hydroxycarbonyl, hydroxycarbonyl C.sub.1-6 alkyl, C.sub.1-3 alkoxycarbonyl, C.sub.1-3 alkoxycarbonyl C.sub.1-6 alkyl, hydroxycarbonyl- C.sub.1-6 alkyloxy, hydroxy, hydroxy C.sub.1-6 alkyl, C.sub.1-6 alkyloxy-C.sub.1-6 alkyl, nitro, cyano, trifluoromethyl, trifluoromethoxy, trifluoroethoxy, C.sub.1-8 alkyl-S(O).sub.p, (C.sub.1-8 alkyl).sub.p aminocarbonyl, C.sub.1-8 alkyloxycarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonyloxy, (aryl C.sub.1-8 alkyl).sub.p amino, (aryl).sub.p amino, aryl C.sub.1-8 alkylsulfonylamino, and C.sub.1-8 alkylsulfonylamino; or two R.sup.1 substituents, when on the same carbon atom, are taken together with the carbon atom to which they are attached to form a carbonyl group; each R.sup.3 is independently selected from the group consisting of hydrogen, aryl, C.sub.1-10 alkyl, aryl--(CH.sub.2).sub.r --O--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r S(O).sub.p --(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --C(O)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r C(O)--N(R.sup.4)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --N(R.sup.4)--C(O)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --N(R.sup.4)--(CH.sub.2).sub.s, halogen, hydroxyl, oxo, trifluoromethyl, C.sub.1-8 alkylcarbonylamino, aryl C.sub.1-5 alkoxy, C.sub.1-5 alkoxycarbonyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl, C.sub.1-6 alkylcarbonyloxy, C.sub.3-8 cycloalkyl, (C.sub.1-6 alkyl).sub.p amino, amino C.sub.1-6 alkyl, arylaminocarbonyl, aryl C.sub.1-5 alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C.sub.1-6 alkyl, hydroxycarbonyl, hydroxycarbonyl C.sub.1-6 alkyl, HC.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--C.tbd.C--(CH.sub.2).sub.t --, aryl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--C.tbd.C--(CH.sub.2).sub.t --, CH.sub.2 .dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--CH.dbd.CH--(CH.sub.2).sub.t --, aryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--SO.sub.2--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--SO.sub.2 --(CH.sub.2).sub.t --, C.sub.1-6 alkoxy, aryl C.sub.1-6 alkoxy, aryl C.sub.1-6 alkyl, (C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, (aryl).sub.p amino, (aryl).sub.p amino C.sub.1-6 alkyl, (aryl C.sub.1-6 alkyl).sub.p amino,

(aryl C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, arylcarbonyloxy, aryl C.sub.1-6 alkylcarbonyloxy, (C.sub.1-6 alkyl).sub.p aminocarbonyloxy, C.sub.1-8 alkylsulfonylamino, arylsulfonylamino, C.sub.1-8 alkylsulfonylamino C.sub.1-6 alkyl, arylsulfonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonylamino, aryl C.sub.1-6 alkylsulfonylamino C.sub.1-6 alkyl, C.sub.1-8 alkoxycarbonylamino, C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, aryloxyearbonylamino C.sub.1-8 alkyl, aryl C.sub.1-8 alkoxycarbonylamino, aryl C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonylamino, C.sub.1-8 alkylcarbonylamino C.sub.1-6 alkyl, arylcarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonylamino, aryl C.sub.1-6 alkylcarbonylamino C.sub.1-6 alkyl, aminocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl).sub.p aminocarbonyl amino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, aminosulfonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminosulfonylamino, (C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino , (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, C.sub.1-6 alkylsulfonyl, C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, arylsulfonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonyl, aryl C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, C.sub.1-6 alkylcarbonyl, C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonyl, aryl C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, C.sub.1-6 alkylthiocarbonylamino, C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, arylthiocarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylthiocarbonylamino, aryl C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl, and (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl; or two R.sup.3 substituents, when on the same carbon atom are taken together with the carbon atom to which they are attached to form a carbonyl group or a cyclopropyl group, wherein any of the alkyl groups of R.sup.3 are either unsubstituted or substituted with one to three R.sup.1 substituents, and provided that each R.sup.3 is selected such that in the resultant compound the carbon atom or atoms to which R.sup.3 is attached is itself attached to no more than one heteroatom; each R.sup.4 is independently selected from the group consisting of hydrogen, aryl, aminocarbonyl, C.sub.3-8 cycloalkyl, amino C.sub.1-6 alkyl, (aryl).sub.p aminocarbonyl, (aryl C.sub.1-5 alkyl).sub.p aminocarbonyl, hydroxycarbonyl C.sub.1-6 alkyl, C.sub.1-8 alkyl, aryl C.sub.1-6 alkyl, (C.sub.1-6 alkyl).sub.p amino C.sub.2-6 alkyl, (aryl C.sub.1-6 alkyl).sub.p amino C.sub.2-6 alkyl, C.sub.1-8 alkylsulfonyl, C.sub.1-8 alkoxycarbonyl, aryloxycarbonyl, aryl C.sub.1-8 alkoxycarbonyl, C.sub.1-8 alkylcarbonyl, arylcarbonyl, aryl C.sub.1-6 alkylcarbonyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl, aminosulfonyl, C.sub.1-8 alkylaminosulfonyl, (aryl).sub.p aminosulfonyl, (aryl C.sub.1-8 alkyl).sub.p aminosulfonyl, arylsulfonyl, arylC.sub.1-6 alkylsulfonyl, C.sub.1-6 alkylthiocarbonyl, arylthiocarbonyl, and aryl C.sub.1-6 alkylthiocarbonyl, wherein any of the alkyl groups of R.sup.4 are either unsubstituted or substituted with one to three R.sup.1 substituents; R.sup.5 and R.sup.6 are each independently selected from the group consisting of hydrogen, C.sub.1-10 alkyl, aryl, aryl--(CH.sub.2).sub.r --O--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r S(O).sub.p --(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --C(O)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --C(O)--N(R.sub.4)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --N(R.sub.4)--C(O)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --N(R.sup.4)--(CH.sub.2).sub.s, halogen, hydroxyl, C.sub.1-8 alkylcarbonylamino, aryl C.sub.1-5 alkoxy, C.sub.1-5 alkoxycarbonyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl, C.sub.1-6 alkylcarbonyloxy, C.sub.3-8 cycloalkyl, (C.sub.1-6 alkyl).sub.p amino, amino C.sub.1-6 alkyl, arylaminocarbonyl, aryl C.sub.1-5 alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C.sub.1-6 alkyl,

hydroxycarbonyl, hydroxycarbonyl C.sub.1-6 alkyl, HC.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--C.tbd.C--(CH.sub.2).sub.t --, aryl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--C.tbd.C--(CH.sub.2).sub.t --, CH.sub.2 .dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--CH.dbd.CH--(CH.sub.2).sub.t --, aryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--SO.sub.2 --(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--SO.sub.2 --(CH.sub.2).sub.t --, C.sub.1-6 alkoxy, aryl C.sub.1-6 alkoxy, aryl C.sub.1-6 alkyl, (C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, (aryl).sub.p amino, (aryl).sub.p amino C.sub.1-6 alkyl, (aryl C.sub.1-6 alkyl).sub.p amino, (aryl C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, arylcarbonyloxy, aryl C.sub.1-6 alkylcarbonyloxy, (C.sub.1-6 alkyl).sub.p aminocarbonyloxy, C.sub.1-8 alkylsulfonylamino, arylsulfonylamino, C.sub.1-8 alkylsulfonylamino C.sub.1-6 alkyl, arylsulfonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonylamino, aryl C.sub.1-6 alkylsulfonylamino C.sub.1-6 alkyl, C.sub.1-8 alkoxycarbonylamino, C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, aryloxycarbonylamino C.sub.1-8 alkyl, aryl C.sub.1-8 alkoxycarbonylamino, aryl C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonylamino, C.sub.1-8 alkylcarbonylamino C.sub.1-6 alkyl, arylcarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonylamino, aryl C.sub.1-6 alkylcarbonylamino C.sub.1-6 alkyl, aminocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, aminosulfonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminosulfonylamino, (C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, C.sub.1-6 alkylsulfonyl, C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, arylsulfonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonyl, aryl C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, C.sub.1-6 alkylcarbonyl, C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, arylcarbonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonyl, aryl C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, C.sub.1-6 alkylthiocarbonylamino, C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, arylthiocarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylthiocarbonylamino, aryl C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl, and (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl; or R.sup.5 and R.sup.6 are taken together with the carbon atom to which they are attached to form a carbonyl group, wherein any of the alkyl groups of R.sup.5 or R.sup.6 are either unsubstituted or substituted with one to three R.sup.1 substituents,

and provided that each R.sup.5 and R.sup.6 are selected such that in the resultant compound the carbon atom to which R.sup.5 and R.sup.6 are attached is itself attached to no more than one heteroatom; R.sup.7 and R.sup.8 are each independently selected from the group consisting of hydrogen, C.sub.1-10 alkyl, aryl, aryl--(CH.sub.2).sub.r --O--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r S(O).sub.p --(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --C(O)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r --C(O)--N(R.sup.4)--(CH.sub.2).sub.s, aryl--(CH.sub.2).sub.r N(R.sup.4)--C(O)--(CH.sub.2).sub.s --,

aryl--(CH.sub.2).sub.r --N(R.sup.4)--(CH.sub.2).sub.s, halogen, hydroxyl, C.sub.1-8 alkylcarbonylamino, aryl C.sub.1-5 alkoxy, C.sub.1-5 alkoxycarbonyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl, C.sub.1-6 alkylcarbonyloxy, C.sub.3-8 cycloalkyl, (C.sub.1-6 alkyl).sub.p amino, amino C.sub.1-6 alkyl, arylaminocarbonyl, aryl C.sub.1-5 alkylaminocarbonyl, aminocarbonyl, aminocarbonyl C.sub.1-6 alkyl,

hydroxycarbonyl, hydroxycarbonyl C.sub.1-6 alkyl, HC.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--C.tbd.C--(CH.sub.2).sub.t --, aryl--C.tbd.C--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--C.tbd.C--(CH.sub.2).sub.t --, CH.sub.2 .dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.3-7 cycloalkyl--CH.dbd.CH--(CH.sub.2).sub.t --, aryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--CH.dbd.CH--(CH.sub.2).sub.t --, C.sub.1-6 alkyl--SO.sub.2 --(CH.sub.2).sub.t --, C.sub.1-6 alkylaryl--SO.sub.2 --(CH.sub.2).sub.t --, C.sub.1-6 alkoxy, aryl C.sub.1-6 alkoxy, aryl C.sub.1-6 alkyl, (C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, (aryl).sub.p amino, (aryl).sub.p amino C.sub.1-6 alkyl, (aryl C.sub.1-6 alkyl).sub.p amino, (aryl C.sub.1-6 alkyl).sub.p amino C.sub.1-6 alkyl, arylcarbonyloxy, aryl C.sub.1-6 alkylcarbonyloxy, (C.sub.1-6 alkyl).sub.p aminocarbonyloxy, C.sub.1-8 alkylsulfonylamino, arylcarbonylamino, arylsulfonylamino, C.sub.1-8 alkylsulfonylamino C.sub.1-6 alkyl, arylsulfonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonylamino, aryl C.sub.1-6 alkylsulfonylamino C.sub.1-6 alkyl, C.sub.1-8 alkoxycarbonylamino, C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, aryloxycarbonylamino C.sub.1-8 alkyl, aryl C.sub.1-8 alkoxycarbonylamino, aryl C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonylamino C.sub.1-6 alkyl, arylcarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonylamino, aryl C.sub.1-6 alkylcarbonylamino C.sub.1-6 alkyl, aminocarbonylamino C.sub.1-6 alkyl, arylaminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, aminosulfonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminosulfonylamino, (C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, C.sub.1-6 alkylsulfonyl, C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, arylsulfonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonyl, aryl C.sub.1-6 alkylsulfonyl C.sub.1-6 alkyl, C.sub.1-6 alkylcarbonyl, C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, arylcarbonyl C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonyl, aryl C.sub.1-6 alkylcarbonyl C.sub.1-6 alkyl, C.sub.1-6 alkylthiocarbonylamino, C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, arylthiocarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylthiocarbonylamino, aryl C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl).sub.p aminocarbonyl C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl, (aryl C.sub.1-8 alkyl).sub.p aminocarbonyl C.sub.1-6 alkyl, and C.sub.7-20 polycyclyl C.sub.0-8 alkylsulfonylamino, wherein any of the alkyl groups of R.sup.7 and R.sup.8 are either unsubstituted or substituted with one to three R.sup.1 substituents, and provided that each R.sup.7 and R.sup.8 are selected such that in the resultant compound the carbon atom to which R.sup.7 and R.sup.8 are attached is itself attached to no more than one heteroatom; R.sup.9 is selected from the group consisting of hydrogen, C.sub.1-8 alkyl, aryl, aryl C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonyloxy C.sub.1-4 alkyl, aryl C.sub.1-8 alkylcarbonyloxy C.sub.1-4 alkyl, C.sub.1-8 alkylaminocarbonylmethylene, and C.sub.1-8 dialkylaminocarbonylmethylene; wherein each p is independently an integer from 0 to 2; each r is independently an integer from 1 to 3; each s is independently an integer from 0 to 3; each t is independently an integer from 0 to 3; and each v is independently an integer from 0 to 6; and the pharmaceutically acceptable salts thereof.

2. The compound of claim 1 wherein W is selected from the group consisting of ##STR21## Y is ##STR22## and Z is selected from the group consisting of ##STR23## and R.sup.1 and R.sup.4 are as defined in claim 1.

3. The compound of claim 2 wherein W is selected from the group

consisting of ##STR24## .

4. The compound of claim 3 wherein W is selected from the group consisting of ##STR25## Z is ##STR26## and each v is independently an integer from 0 to 2.

5. The compound of claim 4 wherein each R.sup.3 is independently selected from the group consisting of hydrogen, fluoro, trifluoromethyl, aryl, C.sub.1-8 alkyl, arylC.sub.1-6 alkyl hydroxyl, oxo, arylaminocarbonyl, aryl C.sub.1-5 alkylaminocarbonyl, aminocarbonyl, and aminocarbonyl C.sub.1-6 alkyl; and each R.sup.4 is independently selected from the group consisting of hydrogen, aryl, C.sub.3-8 cycloalkyl, C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonyl, arylcarbonyl, C.sub.1-6 alkylsulfonyl, arylsulfonyl, arylC.sub.1-6 alkylsulfonyl, arylC.sub.1-6 alkylcarbonyl, C.sub.1-8 alkylaminocarbonyl, arylC.sub.1-5 alkylaminocarbonyl, arylC.sub.1-8 alkoxycarbonyl, and C.sub.1-8 alkoxycarbonyl.

6. The compound of claim 5 wherein R.sup.6, R.sup.7, and R.sup.8 are each hydrogen and R.sup.5 is selected from the group consisting of hydrogen, aryl, C.sub.1-8 alkyl, aryl--C.tbd.C--(CH.sub.2).sub.t --, aryl C.sub.1-6 alkyl, CH.sub.2 .dbd.CH--(CH.sub.2).sub.t --, and HC.tbd.C--(CH.sub.2).sub.t --.

7. The compound of claim 6 wherein R.sup.9 is selected from the group consisting of hydrogen, methyl, and ethyl.

8. The compound of claim 7 wherein R.sup.9 is hydrogen.

9. The compound of claim 5 wherein R.sup.5, R.sup.6, and R.sup.8 are each hydrogen and R.sup.7 is selected from the group consisting of hydrogen, aryl, C.sub.1-8 alkylcarbonylamino, C.sub.1-8 alkylsulfonylamino, arylcarbonylamino, arylsulfonylamino, C.sub.1-8 alkylsulfonylamino C.sub.1-6 alkyl, arylsulfonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylsulfonylamino, aryl C.sub.1-6 alkylsulfonylamino C.sub.1-6 alkyl, C.sub.1-8 alkoxycarbonylamino, C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, aryloxycarbonylamino C.sub.1-8 alkyl, aryl C.sub.1-8 alkoxycarbonylamino, aryl C.sub.1-8 alkoxycarbonylamino C.sub.1-8 alkyl, C.sub.1-8 alkylcarbonylamino C.sub.1-6 alkyl, arylcarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylcarbonylamino, aryl C.sub.1-6 alkylcarbonylamino C.sub.1-6 alkyl, aminocarbonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, (aryl).sub.p aminocarbonylamino C.sub.1-6 alkyl, arylaminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino C.sub.1-6 alkyl, aminosulfonylamino C.sub.1-6 alkyl, (C.sub.1-8 alkyl).sub.p aminosulfonylamino, (C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl).sub.p aminosulfonylamino C.sub.1-6 alkyl, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino, (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino C.sub.1-6 alkyl, C.sub.1-6 alkylthiocarbonylamino, C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl, arylthiocarbonylamino C.sub.1-6 alkyl, aryl C.sub.1-6 alkylthiocarbonylamino, and aryl C.sub.1-6 alkylthiocarbonylamino C.sub.1-6 alkyl.

10. The compound of claim 9 wherein R.sup.7 is selected from the group consisting of hydrogen, aryl, C.sub.1-8 alkylcarbonylamino, aryl C.sub.1-6 alkylcarbonylamino, arylcarbonylamino, C.sub.1-8 alkylsulfonylamino, aryl C.sub.1-6 alkylsulfonylamino, arylsulfonylamino, C.sub.1-8 alkoxycarbonylamino, aryl C.sub.1-8 alkoxycarbonylamino, arylaminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminocarbonylamino, (aryl C.sub.1-8 alkyl).sub.p aminocarbonylamino, (C.sub.1-8 alkyl).sub.p aminosulfonylamino, and (aryl C.sub.1-8 alkyl).sub.p aminosulfonylamino.

11. The compound of claim 10 wherein R.sup.9 is selected from the group consisting of hydrogen, methyl, and ethyl.

12. The compound of claim 11 wherein R.sup.9 is hydrogen.

13. A compound selected from the group consisting of '-[N-(3,4,5,6-tetrahydropyrimidin-2-yl)amino]biphenyl-4-carbonyl-2(S)-phenylsulfonylamino-.beta.-alanine, 3'-[N-(5,6,7,8-tetrahydro-[1,8]napthyridin-2-yl)amino]biphenyl-4-carbonyl-2(S)-phenylsulfonylamino-.beta.-alanine, and 3'-[N-(pyrimidin-2-yl)-amino]biphenyl-4-carbonyl-2(S)-phenylsulfonylamino-alanine, and the pharmaceutically acceptable salts thereof.

14. A pharmaceutical **composition** comprising a compound according to claim 1 and a pharmaceutically acceptable carrier.

15. The **composition** of claim 14 which further comprises an active ingredient selected from the group consisting of a) an organic bisphosphonate or a pharmaceutically acceptable salt or ester thereof, b) an estrogen receptor modulator, c) a cytotoxic/antiproliferative agent, d) a matrix metalloproteinase inhibitor, e) an inhibitor of epidermal-derived, fibroblast-derived, or platelet-derived growth factors, f) an inhibitor of VEGF, g) an inhibitor of Flk-1/KDR, Flt-1, Tck/Tie-2, or Tie-1, h) a **cathepsin K** inhibitor, i) an inhibitor of osteoclast proton ATPase, and j) a prenylation inhibitor, such as a farnesyl transferase inhibitor or a geranylgeranyl transferase inhibitor or a dual farnesyl/geranylgeranyl transferase inhibitor; and mixtures thereof.

16. The **composition** of claim 15 wherein said active ingredient is selected from the group consisting of a) an organic bisphosphonate or a pharmaceutically acceptable salt or ester thereof, b) an estrogen receptor modulator, c) a **cathepsin K** inhibitor, and d) an inhibitor of osteoclast proton ATPase; and mixtures thereof.

17. The **composition** of claim 16 wherein said organic bisphosphonate or pharmaceutically acceptable salt or ester thereof is alendronate monosodium trihydrate.

18. A method of eliciting an integrin receptor antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a compound according to claim 1.

19. The method of claim 18 wherein the integrin receptor antagonizing effect is an .alpha..nu..beta.3 antagonizing effect.

20. The method of claim 19 wherein the .alpha..nu..beta.3 antagonizing effect is selected from the group consisting of inhibition of bone resorption, osteoporosis, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, inflammatory arthritis, viral disease, tumor growth and metastasis.

21. The method of claim 20 wherein the .alpha..nu..beta.3 antagonizing effect is the inhibition of bone resorption.

22. The method of claim 18 wherein the integrin receptor antagonizing effect is an .alpha..nu..beta.5 antagonizing effect.

23. The method of claim 22 wherein the .alpha..nu..beta.5 antagonizing effect is selected from the group consisting of inhibition of restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, tumor growth and metastasis.

24. The method of claim 18 wherein the integrin receptor antagonizing effect is a dual .alpha..nu..beta.3/.alpha..nu..beta.5 antagonizing effect.

25. The method of claim 24 wherein the .alpha..nu..beta.3/.alpha..nu..beta.5 antagonizing effect is selected from the group consisting of inhibition of bone resorption, restenosis, angiogenesis, diabetic retinopathy, macular degeneration, inflammation, viral disease, tumor growth and metastasis.

26. The method of claim 18 wherein the integrin antagonizing effect is an .alpha..nu..beta.6 antagonizing effect.

27. The method of claim 26 wherein the .alpha..nu..beta.6 antagonizing effect is selected from the group consisting of inhibition of angiogenesis, inflammatory response, and wound healing.

28. A method of eliciting an integrin receptor antagonizing effect in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 14.

29. A method of treating or preventing a condition mediated by antagonism of an integrin receptor in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 14.

30. A method of inhibiting bone resorption in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 14.

31. A method of inhibiting bone resorption in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 16.

32. A method of treating or preventing osteoporosis in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 14.

33. A method of treating tumor growth in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of the **composition** of claim 14.

AB The present invention relates to compounds and derivatives thereof, their synthesis, and their use as integrin receptor antagonists. More particularly, the compounds of the present invention are antagonists of the integrin receptors .alpha..nu..beta.3, .alpha..nu..beta.5 and/or .alpha..nu..beta.6 and are useful for inhibiting bone resorption, treating and preventing osteoporosis, and inhibiting vascular restenosis, diabetic retinopathy, macular degeneration, angiogenesis, atherosclerosis, inflammation, inflammatory arthritis, viral disease, and tumor growth and metastasis.

ACCESSION NUMBER: 2000:34557 USPATFULL
TITLE: Integrin receptor antagonists
INVENTOR(S): Duggan, Mark E., Schwenksville, PA, United States
Hartman, George D., Lansdale, PA, United States
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 6040311 | | 20000321 |
| APPLICATION INFO.: | US 1999-362528 | | 19990728 |

study, unclassified); BIOL (Biological study)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)

RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L12 ANSWER 5 OF 14 CAPLUS 

AN 2001:544261 CAPLUS

L12 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 2001:772033 CAPLUS
DN 135:314787
TI Induction of rat liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides, nitrofurans, and **quercetin**
AU Rahden-Staron, Iwonna; Czeczot, Hanna; Szumilo, Maria
CS Department of Biochemistry, Medical University of Warsaw, Warsaw, 02-097, Pol.
SO Mutation Research (2001), 498(1-2), 57-66
CODEN: MUREAV; ISSN: 0027-5107
PB Elsevier Science B.V.
DT Journal
LA English
CC 4-6 (Toxicology)
AB The genotoxic activity of environmental xenobiotics is manifested either in their direct interaction with cellular genetic material or in provoking secondary events, among which reactive oxygen species (ROS) prodn. is a common phenomenon. Both pathways can be mediated by the activity of the cytochrome P 450 monooxygenase system. The authors studied the induction of the **CYP 1A** or CYP 2B monooxygenases in rat liver by the fungicides: thiram, captan, captafol, and dodine and the drugs: nitrofurazone and furazolidone and the plant flavonoid: **quercetin**. A cytochrome P 450 induction assay (CYPIA test) was used. S9 prepd. from the livers of rats treated with the test compds. were used to activate ethidium bromide (EtBr) (**CYP 1A** isoenzyme) or cyclophosphamide (CPA) (CYP 2B isoenzyme) in the Ames test. It was found that among the tested compds., the most potent inducer of **CYP 1A** was furazolidone (3.times. 80 mg/kg). Less potent was thiram (1.times. 100 mg/kg), as well as **quercetin** (3.times. 80 mg/kg), and captafol (1.times. 30 mg/kg). On the other hand, thiram (1.times. 100 mg/kg), captafol (1.times. 30 mg/kg), and **quercetin** (3.times. 80 mg/kg) were most potent in the CYP 2B isoenzyme induction, while furazolidone (3.times. 80 mg/kg), and nitrofurazone (3.times. 80 mg/kg) appeared to be less potent in this respect. Captan and dodine (3.times. 80 mg/kg) did not affect the activity of any of the cytochrome P 450 isoenzymes.
ST liver cytochrome P 450 isoenzyme induction fungicide nitrofuran **quercetin**
IT Fungicides
Genotoxicity
Liver
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)
IT 50-18-0, Cyclophosphamide 1239-45-8, Ethidium bromide
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)
IT 332859-78-6, Cytochrome P 450 1A 334677-51-9, Cytochrome P 450 2B
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(induction of liver cytochrome P 450 isoenzymes **CYP 1A** and CYP 2B by different fungicides and nitrofurans and **quercetin**)
IT 59-87-0, Nitrofurazone 67-45-8, Furazolidone 117-39-5, **Quercetin** 133-06-2, Captan 137-26-8, Thiram 2425-06-1, Captafol 2439-10-3, Dodine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological

DN 135:241436
TI The use of a high-volume screening procedure to assess the effects of dietary flavonoids on human CYP1A1 expression
AU Allen, Scott W.; Mueller, Lisa; Williams, Susanne N.; Quattrochi, Linda C.; Raucy, Judy
CS Puracyp, LLC, San Diego, CA, USA
SO Drug Metabolism and Disposition (2001), 29(8), 1074-1079
CODEN: DMDSAI; ISSN: 0090-9556
PB American Society for Pharmacology and Experimental Therapeutics
DT Journal
LA English
CC 18-7 (Animal Nutrition)
Section cross-reference(s): 13
AB The authors examd. the effects of several agents, including dietary flavonoids, on CYP1A1 expression utilizing a recently developed high-throughput screening system for assessing human cytochrome P 450 (CYP) induction. HepG2 cells, stably integrated with regulatory regions of human CYP1A1, were treated with resveratrol, apigenin, curcumin, kaempferol, green tea ext. (GTE), (-)-epigallocatechin gallate (EGCG), **quercetin**, and naringenin. Of these flavonoids, resveratrol produced the greatest increase in CYP1A1-mediated luciferase activity (10-fold), whereas GTE, apigenin, curcumin, and kaempferol produced 2- to 3-fold increases in activity. Compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), omeprazole, or benzanthracene, where increases in luciferase activity ranged from 12- to 35-fold, these flavonoids exhibited weak agonist activity. The remaining compds., EGCG, **quercetin**, and naringenin, produced negligible effects. Cotreatment of cells with TCDD and GTE, naringenin, and apigenin resulted in 58, 77, and 74% redns., resp., in TCDD-mediated CYP1A1 induction, indicating that these flavonoids exhibit potential antagonist activity toward the aryl hydrocarbon (Ah) receptor. Furthermore, results also suggest that GTE and apigenin possess Ah receptor antagonist and weak agonist activities. Thus, the authors showed that a 96-well plate assay allowing high-throughput screening for P 450 induction in less than 24 h was efficient in detg. the effects of flavonoids on human **CYP1A** expression. Signal-to-noise ratios were low, and well-to-well and replicate variability was below 10%, allowing induction to be easily detected in this system. These features illustrate the reliability and feasibility of this high-vol. screening system for identifying CYP inducers. Furthermore, results produced with the stable cell line were corroborated in HepG2 cells and primary cultures of human hepatocytes, suggesting that stably integrated cell lines harboring enhancer elements of P 450 genes may be highly conducive to high-throughput screening.
ST flavonoid diet CYP1A1 gene screening
IT Gene, animal
RL: ANT (Analyte); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process)
(CYP1A1; high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT Animal cell line
(Hep G2; high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT Tea products
(green, ext.; high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT Liver
(hepatocyte; high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT Flavonoids
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)

IT 56-49-5, 3-Methylcholanthrene 56-55-3, Benzanthracene 73590-58-6, Omeprazole
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT 117-39-5, **Quercetin** 458-37-7, Curcumin 480-41-1, Naringenin 501-36-0, Resveratrol 520-18-3, Kaempferol 520-36-5, Apigenin 989-51-5, (-)-Epigallocatechin gallate
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
IT 61970-00-1, Luciferase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(high-vol. screening procedure to assess effects of dietary flavonoids on human CYP1A1 expression)
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L12 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 2001:300371 CAPLUS
DN 135:235878
TI Chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari: modulations of drug-metabolizing enzymes and antioxidant activity
AU Machala, Miroslav; Kubinova, Renata; Horavova, Pavla; Suchy, Vaclav
CS Veterinary Research Institute, Brno, 62132, Czech Rep.
SO Phytotherapy Research (2001), 15(2), 114-118
CODEN: PHYREH; ISSN: 0951-418X
PB John Wiley & Sons Ltd.
DT Journal
LA English
CC 1-3 (Pharmacology)
Section cross-reference(s): 11
AB A series of homoisoflavonoids and chalcones, isolated from the endemic

tropical plant Dracaena cinnabari Balf. (Agavaceae), were tested for their potential to inhibit cytochrome P 4501A (**CYP1A**) enzymes and Fe-enhanced in vitro peroxidn. of microsomal lipids in C57B1/6 mouse liver. The effects of the polyphenolic compds. were compared with those of prototypal flavonoid modulators of **CYP1A** and the well-known antioxidant, butylated hydroxytoluene. 2-Hydroxychalcone and partly 4,6-dihydroxychalcone were found to be strong inhibitors of **CYP1A** -dependent 7-ethoxyresorufin O-deethylase (EROD) activity in vitro comparable to the effects of **quercetin** and chrysin. The first screening of flavonoids and chalcones of Dracaena cinnabari for antioxidant activity was done in an in vitro microsomal peroxidn. assay. While chalcones were shown to be poor antioxidants, 7,8-methylenedioxy-3(4-hydroxybenzyl) chromane, as one of the tested homoisoflavonoids, exhibited a strong antioxidant activity comparable to that of the strongest flavonol antioxidant, **quercetin**.

ST Dracaena cinnabari homoisoflavonoid chalcone drug metabolizing enzyme antioxidant

IT Dracaena cinnabari
Liver
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT Flavonoids
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT Enzymes, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(drug-metabolizing; chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT Peroxidation
(lipid; chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT Lipids, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(peroxidn.; chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT Antioxidants
(pharmaceutical; chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT 59793-97-4, 7-Ethoxyresorufin O-deethylase
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT 94-41-7D, chalcone, derivs. 117-39-5, **Quercetin** 480-40-0, Chrysin 548-83-4, Galangin 644-78-0, 2-Hydroxychalcone 6665-86-7, 7-Hydroxyflavone 25515-43-9 148461-99-8 148462-00-4 361160-32-9 361160-34-1 361160-37-4 361160-39-6
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(chemoprotective potentials of homoisoflavonoids and chalcones of

AU Helsby, N. A.; Chipman, J. K.; Gescher, A.; Kerr, D.
CS School of Biochemistry, University of Birmingham, Edgbaston, B15 2TT, UK
SO Food and Chemical Toxicology (1998), 36(5), 375-382
CODEN: FCTOD7; ISSN: 0278-6915
PB Elsevier Science Ltd.
DT Journal
LA English
CC 18-7 (Animal Nutrition)
Section cross-reference(s): 1, 14
AB The inhibitory effect of the isoflavonoids **genistein** and equol on cytochrome P 450 activities has been investigated. **Genistein** and equol inhibited the high capacity component of p-nitrophenol (CYP2E1 substrate) metab. in liver microsomes from acetone-induced mice with IC50 values of approx. 10 mM and 560 .mu.M, resp. (cf. diethyldithiocarbamate, IC50, 69 .mu.M). Using human CYP2E1 from a specific expression system (which overcame multienzyme involvement in the rodent system), non-competitive inhibition was also seen with both isoflavonoids. **Genistein** and equol also inhibited the high capacity component of ethoxyresorufin (**CYP1A** substrate) metab. in liver microsomes from .beta.-naphthoflavone-induced mice with IC50 values of 5.6 mM and 1.7 mM, resp. (cf. .alpha.-naphthoflavone, IC50 0.8.mu.M). Using human CYP1A2 from a specific expression system, noncompetitive inhibition was seen with both isoflavonoids. CYP1A1 inhibition offers a possible explanation for the chemopreventive effect of **genistein** against, for example, dimethylbenz[a]anthracene genotoxicity reported in animals but the IC50 values negate the relevance of this specific chemopreventive action at the levels likely to be achieved from the human diet.
ST chemoprevention genotoxicity isoflavonoid; **genistein** genotoxicity chemoprevention; equol genotoxicity chemoprevention; metab inhibition cell line **genistein** equol
IT Animal cell line
(**CYP 1A** and 2E1; inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT Genotoxicity
Metabolism, animal
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT Isoflavonoids
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT Microsome
(liver; inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT 9035-51-2, Cytochrome P 450, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT 446-72-0, **Genistein** 531-95-3, Equol
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)
IT 100-02-7, p-Nitrophenol, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL

(Biological study); PROC (Process)
(inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metab. by the isoflavonoids **genistein** and equol)

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 1998:302020 CAPLUS
DN 129:49167
TI In vitro biotransformation of flavonoids by rat liver microsomes
AU Nielsen, S. E.; Breinholt, V.; Justesen, U.; Cornett, C.; Dragsted, L. O.; Nielsen, S. E.; Breinholt, V.; Justesen, U.; Cornett, C.; Dragsted, L. O.
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SO Xenobiotica (1998), 28(4), 389-401
CODEN: XENOBH; ISSN: 0049-8254
PB Taylor & Francis Ltd.
DT Journal
LA English
CC 1-2 (Pharmacology)
AB Sixteen naturally occurring flavonoids were investigated as substrates for cytochrome P 450 in uninduced and Aroclor 1254-induced rat liver microsomes. Naringenin, hesperetin, chrysin, apigenin, tangeretin, kaempferol, galangin and tamarixetin were all metabolized extensively by induced rat liver microsomes but only to a minor extent by uninduced microsomes. No metabolites were detected from eriodictyol, taxifolin, luteolin, **quercetin**, myricetin, fisetin, morin or isorhamnetin. The identity of the metabolites was elucidated using Ic-ms and 1H-NMR, and was consistent with a general metabolic pathway leading to the corresponding 3',4'-dihydroxylated flavonoids either by hydroxylation or demethylation. Structural requirements for microsomal hydroxylation appeared to be a single or no hydroxy group on the B-ring of the flavan nucleus. The presence of two or more hydroxy groups on the B-ring seemed to prevent further hydroxylation. The results indicate that demethylation only occurs in the B-ring when the methoxy group is positioned at C4' and not at the C3,-position. The **CYP1A** isoenzymes were found to be the main enzymes involved in flavonoid hydroxylation, whereas other cytochrome P 450 isoenzymes seem to be involved in flavonoid demethylation.
ST flavonoid metab liver microsome cytochrome P450
IT Drug metabolism
Liver
Microsome
(in vitro biotransformation of flavonoids by rat liver microsomes)
IT Flavonoids
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(in vitro biotransformation of flavonoids by rat liver microsomes)
IT 117-39-5, **Quercetin** 480-16-0, Morin 480-18-2, Taxifolin 480-19-3, Isorhamnetin 480-40-0, Chrysin 480-41-1, Naringenin 481-53-8, Tangeretin 491-70-3, Luteolin 520-18-3, Kaempferol 520-33-2, Hesperetin 520-36-5, Apigenin 528-48-3, Fisetin 529-44-2, Myricetin 548-83-4, Galangin 552-58-9, Eriodictyol 603-61-2, Tamarixetin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(in vitro biotransformation of flavonoids by rat liver microsomes)
IT 16545-23-6, Xanthomicrol 36950-98-8, 4'-Hydroxy-5,6,7,8-tetramethoxyflavone 80140-31-4, 3',4'-Dihydroxy-5,6,7,8-tetramethoxyflavone
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(in vitro biotransformation of flavonoids by rat liver microsomes)
IT 9035-51-2, Cytochrome P 450, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological

study, unclassified); BIOL (Biological study)
(isoenzymes **CYP1A**, CYP3A, CYP1A2, and CYP2B; in vitro biotransformation of flavonoids by rat liver microsomes)

RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L12 ANSWER 13 OF 14 CAPLUS 

AN 1994:528697 CAPLUS

DN 121:128697

TI Isoenzyme- and species-specific susceptibility of cDNA-expressed **CYP1A** P-450s to different flavonoids


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SO Biochimica et Biophysica Acta (1994), 1205(2), 325-35
CODEN: BBACAQ; ISSN: 0006-3002

DT Journal

LA English

CC 7-6 (Enzymes)



AB The inhibitory and stimulatory effects of 6 flavonoids with distinct hydroxylation patterns on the recombinant and hepatic mouse and human **CYP1A** P 450s were studied. CDNA-expressed mouse CYP1A1 and CYP1A2 differed in their sensitivity to both hydroxylated and nonhydroxylated flavonoids, resp. A comparison between the mouse and human CYP1A2 revealed that .alpha.-naphthoflavone and flavone did not change the benzo[.alpha.]pyrene 3-hydroxylation activity of human CYP1A2 but inhibited its 7-ethoxyresorufin and 7-methoxyresorufin O-dealkylation activities. In contrast, hydroxylated flavonoids increased the

7-methoxyresorufin O-demethylation and acetanilide 4-hydroxylation activities of cDNA-expressed human CYP1A2 and in human liver microsomes. These compds. inhibited the benzo[a]pyrene 3-hydroxylase activity of cDNA-expressed CYP1A1 and CYP1A2s as well as in mouse and human liver microsomes. Hydroxylated flavonoids did not inhibit NADPH-cytochrome P 450 reductase activity but inhibited NADPH-2,6-dichlorophenolindophenol reductase activity in liver microsomes and in microsomes from recombinant Hep G2 cells. Structure-activity relations indicated the importance of OH groups in the 5- and 7-positions on the A ring of the flavane nucleus. These OH groups accounted for the inhibitory potency of chrysin on each of the activities of the expressed P 450s, whereas the presence of a OH group at the 4'-position on the B ring decreased the inhibitory potency of naringenin compared to that of chrysin. The ortho-orientation of a OH group on the B ring was of importance, inasmuch as **quercetin** was more potent than morin as an inhibitor of cDNA-expressed and hepatic microsomal monooxygenases.

ST **cytochrome P450 1A** expression liver flavonoid

IT Flavonoids
RL: BIOL (Biological study)
(cytochrome P 450 1A expression by mouse and human liver response to)

IT Liver, metabolism
(cytochrome P 450 1A expression by, of mouse and human, flavonoids effect on)

IT Carcinogens
(flavonoids effect on cytochrome P 450 1A of human liver in relation to)

IT Microsome
(xenobiotic-metabolizing enzymes of, of liver of mouse and human, flavonoids effect on cytochrome P 450 1A expression in relation to)

IT Gene, animal
RL: BIOL (Biological study)
(CYP1A1, expression of, by human liver, flavonoids effect on)

IT Gene, animal
RL: BIOL (Biological study)
(Cyp1a1, expression of, by mouse liver, flavonoids effect on)

IT Gene, animal
RL: BIOL (Biological study)
(Cyp1a2, expression of, by mouse liver, flavonoids effect on)

IT Molecular structure-biological activity relationship
(cytochrome P 450 isoenzyme-inducing, of flavonoids)

IT Enzymes
RL: BIOL (Biological study)
(xenobiotic-metabolizing, flavonoids effect on cytochrome P 450 1A of mouse and human liver in relation to)

IT 9035-51-2, Cytochrome P 450, biological studies
RL: BIOL (Biological study)
(1A, expression of, by mouse and human liver, flavonoids effect on)

IT 117-39-5, **Quercetin** 480-16-0, Morin 480-40-0, Chrysin 480-41-1, Naringenin 525-82-6, Flavone 604-59-1, .alpha.-Naphthoflavone
RL: BIOL (Biological study)
(cytochrome P 450 1A expression by mouse and human liver response to)

IT 9038-14-6, Monooxygenase
RL: BIOL (Biological study)
(cytochrome P 450 1A-dependent, of mouse and human liver, flavonoids effect on)

IT 9012-80-0, Acetanilide 4-hydroxylase 9037-52-9, Aryl hydrocarbon hydroxylase 9039-06-9, NADPH-cytochrome P 450 oxidoreductase 9068-65-9, NADPH-2,6-dichlorophenolindophenol oxidoreductase 59793-97-4, 7-Ethoxyresorufin O-deethylase 83682-88-6, 7-Methoxyresorufin O-demethylase
RL: BIOL (Biological study)
(flavonoids effect on cytochrome P 450 1A of mouse and human in

relation to)

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